

nature

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Is the world getting naughtier?

CONSIDER the following statements:

Military expenditure by Persian Gulf states has risen fivefold in real terms between 1971 and 1975.

In the past 25 years more than 13,000 tanks have been delivered to Middle Eastern countries.

By the year 1980 the world will be producing 80,000 kilograms of plutonium annually (8 kilograms is enough to make one nuclear weapon).

The People's Republic of China now seems to be launching satellites for military reconnaissance purposes.

About 125,000 scientists and engineers in the United States work on military research and development.

They are just a few of the pieces of information that make up the as-ever impressive compilation of military statistics put out by the Stockholm International Peace Research Institute (SIPRI) in its annual yearbook *World Armaments and Disarmament* (MIT Press and Almqvist & Wiksell; SKr 106). For nearly 10 years now the world has been in SIPRI's debt for providing an endless stream of hard and almost-hard facts on military matters all around the world. It is, of course, difficult to judge what impact this impartial reporting has had on public opinion and political thinking (particularly difficult in the United Kingdom where government is distinctly exclusive and as a consequence public opinion is poorly developed on all defence matters). But the global impact has not been negligible and we must hope that the Swedish government never loses its resolve to continue to support SIPRI.

That said, it must be noted that the character of SIPRI, at least as seen through its yearbook, continues to change (as we have reported on a couple of occasions previously). More and more are personal opinions being expressed in the commentaries. Words like "bonanza", "enormity", "seemingly endless", "frightening prospect" and so on crop up increasingly often. The world as seen by SIPRI seems to be getting continually more naughty and fractious. Of course, there are good grounds for showing concern, particularly at the way in which the Third World is picking up military momentum. But there are three reasons why we believe that SIPRI should steer clear of expressing views.

First, if it can't be detached and neutral, no-one else

can. There are plenty of committed institutions around the world—committed to the environment, world government, disarmament and so on. SIPRI's strength has always been its detachment; others might use its figures in any way they choose but it would simply report. Although there is much populist appeal in the path it now seems to be treading, one suspects there is less respect from the hard-headed in the armament and disarmament community.

Second, although there is really no suggestion at all that SIPRI's figures are influenced by its views, there is a risk that the sort of things it will pay attention to could be so affected. Various forms of environmental warfare are discussed in the yearbook, for instance. For example, it is suggested that the ionosphere-Earth wave guide could be used to generate very low frequency electromagnetic fields to couple into people's alpha rhythms. "If methods could be devised to produce greater field strengths . . . either by natural (for example, lightning) or artificial means, then it may become hypothetically possible to impair the performance of a large group of people in selected regions over extended periods." Now this is the idlest of coffee-table musing with, at first sight, the severest of physical objections to be laid against it. Is it right that SIPRI, in its yearbook, should be mixing such vague ideas with its strong brew of hard facts?

Third, the news is not always as bad as SIPRI makes out. "The arms race between the two great powers continued unabated", says SIPRI. But in real terms, military expenditure in the Soviet Union has held roughly constant since 1968 and in the United States has declined in the same period by more than a quarter (aided, of course, by the withdrawal from Vietnam). The world total of annual military expenditures (which includes burgeoning Middle East figures) has remained on a plateau of 210,000 million US dollars (at 1970 prices) for seven years now, and as a percentage of gross domestic product has accordingly declined steadily. It goes without saying that this is a frightening sum of money, but with growing complexity and thus cost of armaments, constant expenditure means less commitment. SIPRI's own figures seem open to more optimistic interpretations. □



Towards an environmental ethic

How should decisions concerning the environment be made? Eric Ashby, examining recent work on the subject, offers his view

IN 1961 there was published in Washington a report under the modest title House Document 522. It ran to eleven volumes. It was prepared by the American Army Corps of Engineers. Its theme was a Comprehensive Survey of the Water Resources of the Delaware Basin. The report recommended that a dam should be built on the Delaware River at a place called Tocks Island in New Jersey. The dam would create a lake about 37 miles long. The estimated cost (when it was reviewed at 1975 prices) was about \$400 millions.

House Document 522 includes a massive exercise in cost-benefit analysis. The recommendation to build a dam rested on four arguments: to diminish the danger of floods downstream; to provide a water reserve in case of drought; to run a hydro-electric power plant; and to create a recreation area of 72,000 acres which would attract (according to the first estimate) as many as 9 million visitors a year. These potential visitors were regarded as a substantial "benefit" in the cost-benefit analysis, on the assumption that they would be worth \$1.35 per person per recreation day.

Of course the proposal generated massive opposition. Some of the quantified values were challenged, but the most powerful arguments against the proposal rested on unquantified values: desecration of the last free flowing river in the east of the USA; acres of wilderness alienated for roads and parking lots and motels; the plea that this beautiful part of the river should be treated as an endangered species. It is a debate familiar to us in Britain. We had something like it over the Cow Green reservoir in Upper Teesdale in the 1960s.

The dam is not yet built, nor at present is it likely to be built. But the fifteen-year-long controversy over it has produced one admirable and important second order consequence. It prompted the American Academy of Arts and Sciences to sponsor a study of the problems of decision-making about the environment. The prime problem is how to incorporate what the study calls "fragile" values into the "hard"

values which can reasonably be quantified. For over four years a group including lawyers, scientists and philosophers have reflected on this issue. Their conclusions are now issued in a book* which is, I believe, the first serious and authoritative work on the philosophy of environmental protection. Its starting point is the Tocks Island controversy, which is going to be treated intensively in a second volume to be added to this study†. But the reflections go far beyond that specific episode. In essays, all of which are careful and one or two of which are brilliant, the nine authors set out their attitudes to the conflict of values which is the inevitable dilemma facing those who have to make decisions about the exploitation of the environment by man.

The impressive feature of the book is that it does not reach a consensus; it does not offer formulae for decision-makers. Its authors have the courage to declare that there is no simple resolution to the question: how do you reconcile the protection of nature with the needs of industrial man? It eschews slick and glossy utopias. It was J. S. Mill who wrote that the test of good government is the degree to which it increases the sum of good qualities in the governed. All the authors agree that there are values about the environment which are "good" and values which are "bad". But, as Robert Dorfman writes in the last essay, "I do not believe that those questions can be answered definitely, now or ever; but neither can they be ignored. The progress that I see in our project is that it points the way to living honestly with these forever open questions".

In Britain those who make decisions about the environment are no longer philistines. The main difficulty about "soft" or "fragile" values is not that they are neglected in the decision-making process; it is, as Harvey Brooks writes, "bringing them into a common intellectual framework with the rest of the analysis" for the purpose of political decisions, where "hard" quantified data based on cost-benefit analysis is likely to be more easily accepted. One of the redeeming features of the consumer society which has to be put in the balance against its many ugly features is that "fragile" values do play an increasingly important part in political decisions about the environment. In the files of the Department of the Environment there are many examples of planning permissions being

refused because unquantified values were deemed to carry greater weight than quantified ones.

This is encouraging, but it does not dispose of the difficulties which arise when there is a conflict of values, a clash of "goods" and the necessity to make a choice between them. The American Academy has put us all in its debt by facing these difficulties head on, in the book the Academy has sponsored. Among the issues discussed in the book, two seem to me to be particularly important.

- Values can be compared more easily if they can all be expressed in the same units. Does this justify the attachment of a money-tag to all values, even though this means what economists call "shadow pricing" (for example, the "value" of a view of the South Downs is the extra cost of not defacing the view if a road or a line of electric pylons has to be built in the neighbourhood)? Or are the values themselves distorted as soon as you try to put a money-tag on them?

- Is it possible to declare certain clear ends or goals in environmental policy, and to accept these ends as "given"—"axiom values", as it were—so that all decisions about the environment become means toward unalterable values which are the ends?

In a closely argued and subtle essay, Laurence Tribe tackles both these issues. He dismisses one common criticism of cost-benefit analysis of environmental problems: the criticism that to quantify "fragile" values (a view, a rare plant community, a river in its natural state) is *illogical*. All such concerns "can in theory be incorporated into a rigorous analysis . . ." The danger of incorporating them, of putting price tags on elusive externalities, is more subtle. It is twofold. First, it flattens any sense of obligation toward natural objects into an aspect of self interest ("What is its money value to *me*?"). Second, it exerts "an enormous reductionist pressure on all values that would otherwise seem incommensurable with a calculus of human wants." In other words, the very translation of the values into manageable units may deprive them of their significance. Tribe's essay, and others in the book, particularly the essay by Harvey Brooks, are a great encouragement to the decision-maker when he is presented with a tidy and misleadingly comprehensive assessment of some project which will have an impact on the environment, neatly distilled into cost-benefit equations. The decisions he has to make, writes Harvey Brooks, "are fundamentally

*Tribe, L. H., Schelling, C. S., and Voss, J. (Eds.) *When Values Conflict* (Ballinger, Cambridge, Mass., 1976). To be published in the UK by Wiley.

†Feiverson, H. A., Sinden, F. W., and Socolow, R. H. (Eds.) *Boundaries of Analysis* (Ballinger, Cambridge, Mass., 1976). To be published in the UK by Wiley.

political in the sense that they ultimately involve competing or conflicting values, and therefore cannot be resolved by purely 'rational' (that is, empirical and logical-deductive) means."

If this view is accepted, there remains the problem of how values are to be incorporated into analysis if they cannot be quantified, or related to self interest. To the question "Does nature embody values apart from its usefulness in serving man's desires?", the answer seems to be, "Yes, but we are not clear, and cannot expect ever to be clear, what these values are." So Laurence Tribe proposes a fresh attitude to this issue. He concedes that most people are very vague about the values they hold on environmental matters and that "such inchoate values are crystallised into distinct preferences or criteria of choice only through the concrete process of seeking means to attain them and gradually discovering what such means entail." In other words, values evolve through the choices made in groping toward them, and it is an essential aspect of freedom that we can choose what we shall value.

The direction in which this argument leads is as follows. First, there are no sanctified principles upon which environmental decisions rest, no "axiom values"; but all decisions are rationalised in some provisionally held principles, and these provisionally held principles *evolve* in the light of the choices which are made and the observed consequences of these choices. (Consider, for instance, how our values about pesticides have evolved through experience of making decisions about the use of pesticides.) Second, the direction in which principles evolve in our attitude toward the environment is taking us beyond the crude criterion of self interest—witness the recent legislation in Britain to protect some species of wild animals and plants, so rare that not one in a hundred of the legislators is likely ever to have seen any of them! It is this sort of argument which leads Laurence Tribe to

suggest that social values about environmental issues progress in a spiral (their direction depending upon their position in the spiral; a framework for choice, as he calls it, which "must incorporate procedures for its own evolution.") And as a starting point on the spiral, Tribe suggests that we "should avoid a premise of human domination—or indeed a premise of the total subservience of any form of being to any other."

A feeling of obligation toward organisms other than man and a responsibility for protecting natural objects—valleys, forests, wildernesses—is of course no novelty among individuals. It is the codification of this obligation or responsibility which is novel. Nearly ten years ago, for instance, there was a planning enquiry into the effect which a North Sea Gas terminal would have on an area of natural beauty on the Norfolk coast. It was the Minister of Housing and Local Government who had to decide whether the area of natural beauty would be damaged by the siting of the gas terminal. In a word, he had to act as the "guardian" of the area of natural beauty. This attitude to a natural object prompts one to ask whether natural objects, trees and woodlands, creatures other than man, should have "rights". At first sight this seems to be a sentimental and mystical attitude to nature. But that is not the view of some hard-headed practical lawyers. In 1972 an article by a professor of law in California, Christopher Stone, examined the singular thesis: Should Trees have Standing? The article has since been published as a book, together with judgement from the Supreme Court on the legal case which prompted Stone's article†.

The circumstances which brought the action to the Supreme Court were these. The United States Forest Service had granted a permit to Walt Disney Enterprises Inc to "develop" Mineral King Valley in the Sierra Nevada mountains. The decision was

challenged by the Sierra Club, which acts vigorously to defend natural objects in America. The Sierra Club lost its case for a reason which would apply to an analogous case if it were to be brought to the courts in Britain, namely because the Club had no sufficient "personal stake in the outcome of the controversy." But three members of the Supreme Court dissented from this decision, and one of the dissenting judgements, by Mr Justice Douglas, drew its inspiration from Stone's article in the California Law Review. He said: "The critical question of 'standing' would be simplified . . . if we fashioned a federal rule that allowed environmental issues to be litigated before federal agencies or federal courts in the name of the inanimate object about to be despoiled . . . Contemporary public concern for protecting nature's ecological equilibrium should lead to the conferral of standing upon environmental objects to sue for their own preservation."

In his lively essay, Stone examines the way rights in law have been conferred upon children (who have not always had rights in law), women, subject peoples formerly enslaved, and so on. He then reminds us that the "world of the lawyer is peopled with inanimate right-holders: trusts, corporations, joint ventures, municipalities . . ." So there is nothing unreasonable about putting natural objects, as the law puts other non-human things, into the category where their interests can be defended in courts by properly recognised guardians. Of course, before the principle could be adopted, many subsidiary questions (such as: Who should be the guardians?) would have to be settled. But the idea is worth serious reflection for it would be one more step in the evolution of an environmental ethic which does not rest on the assumption that nature is made for man. □

†Stone, C. D., *Should Trees have Standing?* (Kaufmann, Los Altos, California, 1974).

The attack on tropical disease

Alex Dorozynski looks at the World Health Organisation's efforts to relieve man of one of his major burdens

IN spite of recent expressions of concern about the new economic order, interdependence of nations, and co-operation, there is one area of enormous importance to the developing world that has largely been ignored: that of tropical diseases. They affect several hundred million people, represent a permanent human burden, and a major obstacle to development. Tropical disease research has hardly

benefited from the explosion of knowledge in bio-medical sciences that has taken place in the developed world.

In fact, some of the tropical diseases are now recrudescing, because parasites have become resistant to drugs and vectors to insecticides, because agricultural development sometimes contributes to the creation of conditions required for a disease to become endemic, and because international aid

toward the control of tropical diseases has shrunk in the face of increasing costs. In the past year, the World Health Organisation (WHO) has been mounting a new attack on these diseases. The approach is novel, and the outlook promising, although one major element of uncertainty still remains: will there be enough money to carry out this long term programme?

The global annual investment in tropical disease research is estimated by the WHO at about \$30 million, which is a mere pittance of money in comparison to huge budgets devoted to other areas of bio-medical research. Advances in treatment and control of

tropical diseases have been painstakingly slow, in spite of the fact that most of the causative agents are relatively simple parasites belonging to the lowest orders, protozoa or worms, that in most cases are completely incapable of independent life and rely for survival on the highly specialised environment provided by two or three or sometimes more successive hosts.

"According to the law that viability is inversely proportional to the stringency of ecological requirements," says Professor Christian de Duve, "the pathogenic parasites certainly occupy the bottom of the scale. They are puny enemies indeed." He is the director of the International Institute of Cellular and Molecular Pathology, Brussels, and a member of Rockefeller University, New York. A consultant of the WHO in the new "Programme of Research and Training in Tropical Diseases", he is one of the scientists who has suggested a new approach to tropical diseases research in which the basic biology of the parasite becomes the prime target.

The diseases

Six diseases have been selected as the initial targets of the WHO's Special Programme: malaria, schistosomiasis, trypanosomiasis, filariasis, leprosy, and leishmaniasis.

● Malaria is one of the most widespread diseases in the world, affecting some 200 million people. In Africa, about one-fourth of all adults suffer from malarial fever at one time or another, and at least one million children die of the diseases every year. In several areas of Asia and South America, *Plasmodium falciparum* (one of the four human malaria parasites, responsible for the severest forms of the diseases), has developed resistance to 4-aminoquinolines, major anti-malarial drugs. In Asia resistant strains have progressed subward to reach the Indo-Pakistani subcontinent, and they represent a great threat to Africa, where *P. falciparum* is the main malaria parasite.

In addition, mosquitoes have developed resistance to some insecticides and, as a result of increased costs and reduced international assistance programmes, many countries cannot afford to carry out intensive anti-malarial campaigns.

India is an example. The number of cases there of malaria had been reduced to a low of 60,000 in 1962, but last year it exceeded four million (a 70-fold increase). In Africa, there appears to be no hope of malaria control in the near future. Dr Adetokunbo O. Lucas, former president of the Nigerian Medical Research Council, who in April became the director of the WHO Special Programme, points out that

several intensive pilot projects have shown that infection is so deeply entrenched in the environment that spraying of insecticides and drug distribution are not sufficient to interrupt transmission.

In some parts of the world, malaria has inadvertently been promoted by irrigation. A typical example is that of the Gezira, the region between the White Nile and the Blue Nile south of Khartoum. Irrigation had turned the Gezira into the most densely populated and most prosperous agricultural region of Sudan, and the periodical drying out of peripheral irrigation ditches, possible when the area was almost exclusively planted to cotton, had to be abandoned when other crops (notably, rice) were introduced. In 1950, an outbreak of malaria affected more than half of the total labour force, and since then malaria has been endemic. A major effort is now under way to keep the diseases under control.

● Schistosomiasis, caused by trematodes (or fluke worms) also affects about 200 million people. Free-swimming larvae (or cercariae) penetrate through the skin of a person entering the water, migrate to the liver where they mature into adult worms that settle inside blood vessels and go on laying eggs for several years. Infected people may become emaciated, with an enlarged spleen and bloated abdomen, and many develop cancer. It is possible to treat individual cases with injections of antimonials and other compounds, but most of these can cause severe side effects. There is no adequate method for mass treatment.

Schistosomiasis is often associated with rural development. Thus the incidence of the disease has been increasing in Egypt and Sudan since the creation of Lake Nasser, and in Ghana since the construction of the Akosombo Dam on Lake Volta. In Northern Nigeria there has been a three-fold increase in three years around the lake created by the Kainji dam, and even in the semi-arid Arabian Peninsula irrigation has introduced schistosomiasis to regions where it did not previously exist.

● Filariasis affects about 300 million people. The two major filarial parasites, *Wucheria bancrofti* and *Brugia malayi*, transmitted by mosquitos, infect about 250 million people (mainly in Africa, the Indian subcontinent, South-East Asia, the Philippines, China, and the Pacific Islands). They cause elephantiasis of the legs, arms and genitals, by obstructing the flow of lymph.

One form of filariasis is onchocerciasis (or river blindness), caused by *Onchocerca volvulus*, transmitted by *Simulium damnosum*—the blackfly. In the upper basin of the Volta river, more than a million people are in-

fected and thousands are blind. A costly control programme has been initiated in seven of the most affected countries; it involves the spraying from helicopters of the often inaccessible fast-flowing streams where the blackflies hatch, and the treatment of infected persons, but there is a need for better drugs, with less side effects and more effective against adult worms.

● Trypanosomiasis affects some 10 million people. The African form of the disease, sleeping sickness, is endemic over about 10 million square kilometres of land, much of it fertile but abandoned to the tsetse fly, vector of the disease. (It is estimated that this land could provide for a cattle population of 125 million.) The South American form of trypanosomiasis is Chagas' disease, incurable and fatal (it destroys the heart).

● Leprosy, one of the most perplexing infectious diseases, also affects at least 10 million people. Although Hansen's bacillus, or *Mycobacterium leprae*, was described more than a hundred years ago, it has not been grown *in vitro* (or, at least, no reported culture has yet been confirmed). Even the mode of transmission of the disease is not well understood, and although it is generally believed that infection is from man to man, Hansen's bacillus has also been identified in insects, and some researchers believe their bite may also be involved.

● Leishmaniasis, the sixth disease included as a target in the initial stages of the Special Programme, is caused by various protozoa of the genus *Leishmania*. Two of its forms are lethal: the South American *espundia*, which kills by destroying the face, and visceral leishmaniasis, or kala azar.

The programme

There are several reasons why the timing for a renewed effort to develop remedies against these diseases is propitious.

The means to control and treat them are limited, and there is relatively little research directed towards new remedies. A number of recent findings, however, both in parasitology and fundamental biology, can be used to explore new approaches. Several such approaches have been identified, and "task forces", or scientific working groups, have been formed by the WHO, consisting of top scientists in various disciplines, to pursue specific goals.

One group, for instance, is concerned with immunology of malaria, and its ultimate goal is to develop reliable, long-acting vaccines, which would undoubtedly revolutionise the control of malaria. Infection has long been known to produce a certain degree of immunity, and some experimental vaccines have already been pro-

duced. Last year, it was shown that some people possess a genetic factor that prevents the invasion of red blood cells by malaria parasites. This genetic trait conferring resistance to malaria is called "Duffy negative", because red cells lack the Duffy antigen, named after the first person in whom it was discovered. It appears that this antigen leads the parasites into the red blood cells, and that in Duffy negative people the absence of the antigen protects the blood cells against the parasites. Some preliminary experiments indicate that certain enzymes can block the antigen, thus conferring anti-malarial protection to the cells.

An even more important finding was announced only last April by Professor William Trager of the Rockefeller University in New York, who is a member of the Special Programme's "task force" on immunity to malaria: he has succeeded in maintaining a laboratory culture of *Plasmodium falciparum* for three months. This is the first time that a continuous culture of any of the malaria parasites has been successfully established *in vitro* and gone through a large number of multiplication cycles. Large amounts of pure *P. falciparum* can now be available, and this is a significant step towards the development of a vaccine.

The harvest of a large number of *Mycobacteria leprae* is also possible since researchers in Louisiana found in 1971 that injection into nine-banded armadillos caused massive infection of the animals. (Previously, infection had been achieved only in mouse footpads, and it was very limited.)

The WHO's scientific working group on immunology of leprosy has already engaged in antigen preparation and purification, and three promising antigens have been isolated, one in Venezuela, one in the United Kingdom, and one in the USA. As with other task forces, the specialists from several countries meet periodically, either at the WHO headquarters in Geneva or elsewhere, to coordinate their efforts. There is good hope that a practical vaccine, providing long-term protection against *M. leprae* can be developed, and a tentative schedule has already been established: if all goes well, field trials should start in four or five years.

Less is known of the immunological aspects of schistosomiasis, filariasis, trypanosomiasis and leishmaniasis, but there is, nevertheless, hope that vaccines can also be developed against at least some of these diseases.

Recent research has shown, for instance, that the *Schistosoma* parasite has a very sophisticated system to ensure its survival in spite of the immunological defences of the organism it invades. When a free-swimming pre-adult cercaria penetrates through the

skin of a person, it does trigger antibody production. But within a few days, by the time the parasite reaches maturity, it appears to cover its antigens with proteins that mimic the host's own antigens. Thus, if immunisation could increase the organism's reaction against the pre-adult cercaria, the schistosoma could be destroyed before it had time to protect itself.

High technology

At the same time, other participants in the Special Programme are exploring the possibility of entirely new approaches, relying on recent bio-medical knowledge. Professor de Duve, for instance, is attempting to apply "lysosome therapy" to some of the tropical diseases. "Lysosomes," he says,

are essentially miniature little stomachs, which occur in all cells. One of their principal functions is to serve in the digestion of food 'eaten' by the cells by a special capture process, called phagocytosis or pinocytosis, depending on the size of the food particles taken in. As with humans, cells may be greedy or frugal, and they may exhibit a wide variety of tastes. Our purpose is to take advantage of these differences to kill certain cells selectively by poisoning their favourite food. To do this, we bind the poison to a carrier molecule in such a way that it will be released when it gets in the lysosome, that is, in the stomach of the cells that have eaten this poisoned food.

Experiments in mice carried out at the International Institute of Cellular and Molecular Pathology in Brussels have shown that such "selective poisoning" can be effective against at least one *Trypanosoma* parasite. A similar approach can be tried for the treatment of diseases such as schistosomiasis or onchocerciasis, caused by worms with very tough skins, whose weak points may be their "stomachs". Even the leprosy bacillus, which has no lysosomes of its own, may be susceptible to similar "poisoning" because it develops within membrane-bound vacuoles resembling lysosomes in the macrophages of its host.

Enzyme therapy is another possible approach. It is known, for instance, that fertilised eggs of the nematode worm, another parasite of man, manufacture chitin, a substance that protects the cell; since mammals do not produce chitin, interference with the enzymes needed to produce it could destroy nematode eggs without affecting the human host.

Interference with the parasites' nervous system could also be explored—notably by acting on chemotaxis, an important determinant of behaviour, or on neurotransmitters (experiments with *Caenorhabditis elegans*, a free-living soil nematode, have shown that tetra-mysole, an agonist of acetylcholine, a major neurotransmitter, effectively paralyzes the worms, arresting feeding

and mobility).

Vector control, likewise, can benefit from goal-oriented research, notably in the area of biological vector control. It is known that parasites can be infected by other parasites (for instance, mermithid worms can infect and kill the tsetse fly). But basic research into the biology of the insect and its predator, and into the ecological impact of such an approach, must first be carried out.

These are "high technology" approaches, that require the commitment of skilled manpower and adequate budgets. Their goal is the development of remedies on the basis of knowledge of parasite function and biology, rather than through often empirical, large-scale screening, such as has been widely used until now in anti-parasitic drug research.

The purpose of the Special Programme of Research and Training in Tropical Diseases is not, however, limited to such research, much of which must be carried out in institutes, universities, and pharmaceutical laboratories in the developed world. The very countries most affected by tropical diseases will be associated with the programme.

At the beginning, the focus will be on Africa, which has the highest prevalence of the six diseases and where multiple infection is almost the rule. Several African countries have expressed their willingness to participate in and contribute to the programme, and Zambia has offered laboratories in the large Ndola hospital complex for the creation of a multidisciplinary research centre bringing under the same roof different aspects of laboratory research and clinical medicine. Strengthening of existing laboratories, creation of new ones if necessary, and training of additional indigenous personnel, are planned as part of the programme to promote self-reliance in the very parts of the world where tropical diseases are prevalent.

Several meetings devoted to the Special Programme, co-sponsored by the WHO and the United Nations Development Programme, have already been held, and scientific work has started with the help of initial funds contributed last year by several nations and organisations. But a long-term commitment is needed, as the programme cannot be financed from the shrinking budgets of the WHO or UNDP. This is why a meeting with potential donor nations and agencies will be held later this year. In view of the magnitude of the problem being tackled, and the results that can be expected, the initial annual budget of \$15-\$20 million (the price of a jet fighter or a few miles of superhighway) seems reasonable enough. □

USA

Assessing the OTA

Congress's three-year-old Office Technology Assessment (OTA) has come under sharp, and potentially damaging, criticism from another Congressional committee and from the former chairman of its own advisory council. Both have argued that it has failed to live up to expectations and that there is still no clear understanding of its role and function. Colin Norman reports from Washington.

ESTABLISHED in 1972 by an Act of Congress, OTA is meant to furnish Congress with advice and analysis concerning scientific and technological issues. Its mission is frequently described as providing an early warning system on such matters. But its chief problem, which lies at the root of much of the criticism, is that on the one hand it is expected to study long-term issues, while on the other it is supposed to help Congress, which is more concerned with yearly budgets and two-yearly elections.

OTA studies and reports on matters referred to it by other committees of Congress, and it also generates some studies itself. Its operations are managed by a Director, Emilio Q. Daddario, a former Congressman who wrote the original legislation which led to OTA's establishment, and its policy is provided by a board consisting of

six Senators and six Congressmen. In addition, an advisory council, whose members are drawn from industry and academia, provides advice on OTA's operations.

The first criticism of OTA's operations surfaced in the office's recently-published annual report, which contained a letter from Harold Brown, President of Caltech, resigning as chairman of OTA's advisory council, and a response from Representative Olin Teague, chairman of the office's governing board.

Written last December, the letter begins with some words of praise for a few OTA studies, but criticises the fact that the office has taken on too many trivial tasks and asserts that "few of us on the council, I believe, would say that we are satisfied with what has been accomplished, compared with what we hoped for and still believe possible". Brown suggests in particular that OTA has been concentrating too much on immediate problems: "inevitably there are strong pressures on the Congress as well as on the Executive Branch to concentrate on immediate problems. Certainly those problems must be faced as they arise. But there needs to be a balancing effort within the Congress to foresee problems of the medium and even the long term future".

Less gentle criticism has come from

an obscure Congressional body, known as the House Commission on Information and Facilities, chaired by Representative Jack Brooks of Texas. On the basis of an 8-month study, the commission last week issued a report which concludes that "OTA remains substantially short of reaching levels of performance reasonably expected of an information resource of its size and cost and access to expertise". The report, in short, suggested that OTA has been beset by operational problems and by lack of a clear definition of its functions.

The report states that the Commission found confusion among OTA's staff, council and board, and between OTA and some Congressional committees, over the office's role and responsibilities. It suggests, therefore, that OTA's statutory authority should be reviewed, and a clear definition of technology assessment should be drawn up, presumably so that OTA's territory is staked out and so that there's no overlap of its functions with those of the Congressional Research Service or the General Accounting Office. The report goes on to state, however, that so far there has been no such overlap.

More specifically, the report expresses reservations about the fact that OTA has been performing a growing share of its studies itself, rather than having them done by outside contractors, and suggests that OTA should have a firmer policy on which kinds of studies should be performed in-house. That criticism conflicts, however, with a comment made by Brown in his letter. Listing some "substantial advances" made by OTA, Brown notes that "an initial tendency to think almost solely in terms of contracted studies has been succeeded by a more balanced procedure involving advisory panels, contracted studies, and some (as yet rather little) in-house assessment work".

But perhaps the Commission's most biting criticism concerns OTA's administrative structure. "Organisationally", it says, "OTA lacks the minimum of orderly structure", and it suggests that OTA should begin immediately, if necessary with the help of management consultants, to put itself in order.

Finally, both the Commission and Brown criticise the poor relations which have developed between OTA and its advisory council. "At one time or another", Brown states, "most Council members have expressed frustration about the relatively large amount of time, effort and persistence that they have invested in terms of the effect they feel they have had. I believe that the important task of strengthening communications between the

NSF budget goes to conference

HOPES for at least a modest increase in funds for basic research in the United States have been unexpectedly revived by the Senate. After intensive lobbying from scientific and higher education organisations, the Senate last week restored most of the money which the House of Representatives had slashed from the budget of the National Science Foundation (NSF), and the matter must now be decided in a conference committee consisting of members from each body. It's a fair bet that NSF will end up with a small increase, though not as great as the 20% boost proposed by the Ford Administration.

President Ford's budget request for NSF for the fiscal year which begins on October 1 was designed to offset the effects of inflation, which has eaten deeply into support for basic research over the past few years. But the House slashed nearly \$60 million from NSF's budget, giving

the agency less than a cost-of-living increase, largely on the grounds that basic research isn't too badly off in relation to other items in the federal budget.

The House's parsimony prompted a massive letter-writing campaign to key Senators, and a move to restore the funds, led by Senators Charles Mathias of Maryland and Edward Brooke of Massachusetts, proved successful. The senate agreed to Mr Ford's proposed 20% increase, in spite of opposition from Senator William Proxmire, Chairman of the Senate subcommittee which handles NSF's budget request. Proxmire said that he believes the increase is much too generous, but was outvoted in the committee.

The conference committee will probably settle on a figure about half way between the levels approved by the House and Senate, which would at least give basic research its first real increase in about five years.

Board and the Council needs to be faced during the coming year". And the House Commission on Information and Facilities, noting that there has been considerable confusion over the role of the council, suggests that "substantial revision of the Council's statu-

tory function, or its abolition, be proposed on grounds that unless Council contributions are integrated more effectively in the policymaking process, its existence can only be a source of frustration and disharmony within the agency".

There is clearly going to be more debate before OTA is able to establish a comfortable role in which it acts as a long-term analyst and at the same time provides advice geared to Congress's short-term outlook. □

EEC

European science policy sought

The European Commission recently unveiled proposals for a Community science policy. Progress towards the aim of a common approach, reports Chris Sherwell, may prove to be more pedestrian than the enthusiasts are prepared to admit.

If the expressions of satisfaction emanating from the vicinity of the Berlaymont Building in Brussels are anything to go by, Europe is now well on its way towards forging a science and technology policy for itself. The Commission of the EEC, which has its headquarters there, recently unveiled the results of a closed symposium which took place under its auspices in Milan towards the end of May.

The aim of the symposium was to gather suitable suggestions for guidelines regarding research and development which the Commission might present to the relevant Council of Ministers later this year. This it reckons to have achieved. Among the 100-odd participants were members of the European and the various national parliaments; members of the Commission and of the Economic and Social Committee (a consultative body serving the EEC and Euratom), and government officials; and the customary host of scientists, engineers, industrialists and trade unionists who help to make meetings like this more models of organisation than of representativeness.

The three-day symposium, at Milan's International Institute for Management of Technology, was organised by Directorate General XII (Research, Science and Education) of the Commission with the aid of CERD (the European Research and Development Committee), which is an independent 21-member body of scientists and engineers established by the Community in 1973 to advise the Commission on the formulation of a common science policy. It took place in the context of an effort finally launched two years ago to sow the first seeds of a revamped Community science and technology policy to replace the old sectoral approach of the previous sixteen years.

That effort in fact had its real beginnings many years ago, but it was

only in January 1974 that the Council of Ministers found itself able to give expression to sentiments voiced at the Paris Heads of Government meeting in October 1972; the Council passed four resolutions which would provide a basis for a broad Community science and technology policy. The resolutions covered matters like coordination, participation in the European Science Foundation and programmes of action. The Commission was landed with the task of looking at the science policies of the nine member states with a view to producing Community-wide projects and a common approach externally.

The hope was that the terms of a full-blooded European science policy could be finalised by the end of 1976. The Council, when it met again in June 1975, urged that discussions on the objectives of such a policy be held "without delay", and the Milan symposium, being the major part of those discussions, heralded the end of this first phase in the new Community approach. The next phase begins if and when the Council approves the recommendations of the symposium's five working parties, each of which aimed to tackle separate areas of interest.

Recommendations

The Commission document summarising the working parties' recommendations, taken as a whole, does not make exciting reading, being littered throughout with empty phrases characteristic of all ostensibly agonising searches for lowest-common-denominator agreements. Hidden in the interstices of the Commission's denatured language, however, is some sort of basis for the optimism now being expressed so expansively by the Director General at DGXII, Herr Gunter Schuster. Here, in essence, is the gist of the recommendations.

Working Party I: Long term objectives and priorities. The group recommends that a suitable instrument "such as proposed in 'Europe+30'" be established "at the earliest possible time", and that in the meantime a "small unit of specialists" be set up in or be linked to DGXII "without delay". Lord Kennet from Britain dissented on the latter point, arguing that such a staff

should be actually in DGXII to avoid the danger of it becoming a substitute for Europe+30.

Among the many areas of high priority for research in the long term, the working party includes Europe's ecological system, climatic changes, water management and food shortages.

Working Party II: Medium term objectives and priorities. The main theme informing this group's recommendations emphasises the need to do more to bring innovations to potential customers. The group wants to "make operational, within the Communities (the EEC, the European Coal and Steel Community and Euratom), structures for securing and examining research and development proposals coming from any public or private European organisation". It also wants "to re-launch the Community development contract procedure" reserved for *European* [group's italics] groups of enterprises and multinationals, and "to establish structures for conveying the Commission's intentions". A consultative committee for industry is suggested.

More specifically, the group hopes that certain subjects now neglected or insufficiently developed will receive "special consideration"—among them, hydrographical problems arising out of the existence of multinational basins, European epidemiological research, recycling and reclamation, basic biological research, and ethical problems in genetic matters.

Interestingly, there is a frank acknowledgment of the constraints within which such a policy can work. The human and financial resources the EEC has at its disposal, the group says, "are not considerable", and are so concentrated as to prevent the needs of a real European policy being met over a wider field—and in certain fields, the group declares, the EEC could not be satisfied with the role of a mere catalyst. The scientific and technical activity of the EEC, it stresses, is "an essential part of a true European economic community", and it expresses its hope for a separate budget that would allow further progress.

Working Party III: Coordination of national policies. After recognising that a common policy can only be built up "slowly and step by step out of the coordination of national policies

and common sections", this group deals with coordination in four fields.

In basic science, it says, "the self-coordination of scientific groups, institutes, organisations and associations is the most important and useful instrument of coordination"—and here the group has in mind the cooperation already operating between Britain's SRC, France's CNRS, Germany's Max Planck Institutes, and so on. Where research is carried out almost totally by industry, coordination should be limited to the "harmonisation and coherent development of support activities in the member states".

Research and development for "public services and tasks", on the other hand, "is undoubtedly the central object for coordination in the Community", because in these spheres the national government have sufficient responsibilities. Finally, in the mixed sector ("the most difficult sector for coordination"), where it is noted that funds in all countries are heavily concentrated in a few areas (nuclear, space, electronics), the group states rather vacuously that projects performed by industry but financed by government "should not be excluded from coordination".

Working Party IV: Innovation policy.

Industrial enterprise, says this group, is "the main actor in the innovation process", but it is governments which have to create a favourable climate—and promotion of innovation at the Community level "is at present hampered by the lack of a common industrial policy", which it calls "a serious obstacle to the development of comprehensive industrial R & D programmes".

The Community, it says, can make "an important contribution" towards creating the right climate: among other things, it could support co-operation in long term industrial research and development (fusion, aerospace, solar energy), finance demonstration projects, make more

accessible those public markets where state agencies are large buyers of technology, and improve access for small and medium sized industries to technological innovation. The group, like the second working party, recommends that the Commission set up an industry consultative committee to give industrial viewpoints on the Community's R&D policy.

Far from judging the present economic plight of European countries a deterrent to innovation, the group rather believed this problem called for a special effort. Similarly, state participation in the field was less a constraint than a stimulus, although state encouragement of specific projects—not so much in fields of obvious community interest (energy supply, public transport) as in fields like cybernetics—demanded fundamental discussion. The general feeling was that there was insufficient coincidence between the long term projects of member states and those of the Community—and that the liaison now lacking could, unsurprisingly, only be promoted through greater coordination. Plainly, though, the overlap with Europe's much-sought-after industrial policy is regarded as vital to the success of any research policy relating to industry.

Working Party V: Dissemination and exploitation of results.

The group recommended that "a readily and selectively accessible dissemination system for information about R&D" be set up within the Community, based on national information services and existing contacts between organisations. It further recommended that the Community's four-establishment Joint Research Centre (JRC) should have greater involvement in the exploitation of its own inventions, and that the Community should be able to provide companies with incentives where necessary (through exclusivity or lead times, for example). A small team of experts should also be available, with close links to scientists in the JRC and contractors, on the one hand, and to exploitation organisations in member states on the other.

Long way to go

All of which suggests that there is a long way to go before a real European policy is actually in operation. Although the recommendations reveal a rather limited number of specific institutional proposals, the general disposition in favour of a European approach, and one buttressed through additional budgetary support at that, shines through. The main emphasis, naturally enough, is on the key-word "coordination", perhaps the most crucial block in the fragile Community science edifice. But the question of how best this might be achieved,

although begged throughout, does not receive a great deal in the way of answers.

Not that Europe's welter of institutions does not already include a goodly proportion of science-oriented bodies equipped to supply the answers. Apart from CERD, the most important is probably CREST, the Scientific and Technical Research Committee. It is the product of the January 1974 Council resolution on coordination, and is blessed with the task of "helping Community institutions to define objectives and securing the development of a common policy for science and technology." According to Working Party III, it is CREST which is to be regarded as the central committee where "coordination in all fields of R&D is focused", and Community officials looking to CREST to translate proposals into reality do point to its achievements—in the implementation of Community projects on new energy sources and scientific information, for example, and in planning cooperation with non-Community countries, which it does within the context of COST, the Committee of Senior Officials in Scientific and Technical Research.

But there is little question about how much has really been achieved so far. No one at the Commission doubts that progress has been and will continue to be slow, or that CREST has yet to come into its own. There are pilot projects in hand, in the fields of energy, medical research and the determination of research and development indicators, and these represent practical attempts at coordination and alignment. For the moment, however, they merely supplement existing instruments of coordination, which also include the Advisory Committees on Programme Management (ACPMs).

The perspective being adopted in Europe is thus distinctly medium to long term—CERD itself has proposed that a European Science Year be organised in 1978 or 1979, just before the United Nations World Science Year in 1980. In places the Commission's document makes a tilt towards the themes of humanising science and of Third World development, and there does seem to be an underlying view which argues that, if a European science policy is to have any chance of success at all, then somehow science itself will have to be brought closer to the proverbial man in the street, and be made to be seen as relevant to his needs. But if that laudable view is scuttled by the trials of actually identifying Europe's research requirements, and of formulating an acceptable policy (particularly regarding the distribution of its benefits and its burdens), there should be few surprised faces. □



Herr Schuster, Director General at DGXII.

news and views

Factor VIII

from D. E. G. Austen

FACTOR VIII activity is essential for normal blood clotting and haemostasis, and an inherited deficiency of this activity occurs in haemophiliacs and patients with von Willebrand's disease. In von Willebrand's disease there is also a deficiency of a closely related substance called factor VIII-related antigen (Zimmerman *et al.*, *J. clin. Invest.*, **50**, 244; 1972). The presence of this antigen is demonstrated by raising heterologous antibodies in animals to factor VIII preparations and using them in immunoelectrophoretic procedures. Precipitin lines reveal the presence of factor VIII-related antigen in the plasma or serum of both normal people and haemophiliacs, but not in the plasma or serum of patients with severe von Willebrand's disease.

Haemophilia is determined by an X-linked recessive mechanism of inheritance while von Willebrand's disease is inherited as an autosomal dominant condition. As far as is known, there is no inherited deficiency of antigen alone and the concentration of antigen in normal people and most patients with von Willebrand's disease is roughly proportional to their factor VIII activity. These various relationships between factor VIII and the antigen suggest that their production *in vivo* proceeds by a common route at some stage.

Subunits

Factor VIII is a glycoprotein and many workers have reported it to have a molecular weight of more than 10^6 as determined by gel filtration chromatography. It is relatively easy to bring about an apparent reduction in this molecular size using high or low ionic strength (Weiss *et al.*, *Thromb. Diath. haemorrh.*, **27**, 212; 1972; Owen and Wagner, *ibid.*, **27**, 502; 1972) or reducing agents (Austen, *Br. J. Haemat.*, **27**, 89; 1974) and the factor VIII activity then appears by gel filtration to have a molecular weight of around 230,000. It has been suggested recently, however, that experimental conditions affect the speed at which low molecular weight factor VIII passes through the gel filtration column (Switzer and McKee, *J. clin. Invest.*, **57**, 925; 1976) and so the exact values of molecular weight should not be emphasised at this stage. Factor VIII-related antigen remains unchanged by variations in ionic strength or by very mild reducing conditions. As a result, gel filtration of

the products of such reactions separates factor VIII activity from the antigen, although the extent of this separation is still a matter of discussion. Two different hypotheses have been suggested to explain these results. The first is that factor VIII is made up of several subunits which can be disaggregated, while at the same time, factor VIII-related antigen circulates as a completely separate molecule. The second possibility is that factor VIII consists of a small active molecule which is bound as a passenger to a large carrier molecule of factor VIII-related antigen and that disaggregation experiments simply separate the two.

Several workers have now also cleaved factor VIII-related antigen into subunits. Some have effected this in moderate reducing conditions and retained the antigenicity of the protein (Austen *et al.*, *Nature*, **253**, 55; 1975; Peake and Bloom, *Thromb. Haemostasis*, **35**, 191; 1976). Others have used severe conditions along with sodium dodecylsulphate (SDS) gel electrophoresis and report that they can detect the protein using staining techniques (Marchesi *et al.*, *J. clin. Invest.*, **51**, 2151; 1972; Legaz *et al.*, *J. biol. Chem.*, **248**, 3946; 1973; Switzer and McKee, *J. clin. Invest.*, **57**, 925; 1976). In both cases the molecular weight value obtained is close to that of the small active factor VIII fragment and this may suggest that the antigen and factor VIII are two proteins with a similar basic structure.

Antibodies

Another field of useful investigation has been concerned with the development of factor VIII antibodies. In patients suffering from haemophilia, antibodies can develop to infused therapeutic concentrates of the factor. Such antibodies will inactivate factor VIII both in its high molecular weight or its low molecular weight form. They are non-precipitating and are not believed to cross react with factor VIII-related antigen. But with severely affected von Willebrand's patients who

have a minimal level of antigen the precipitating-type of antibody can develop, as Manucci *et al.* show (page 141 of this issue of *Nature*). These are directed against factor VIII-related antigen and have only a low level of cross reaction towards factor VIII activity.

More information can be obtained by studying antibodies produced in rabbits after infusion of factor VIII and the antigen. Here there is general agreement that antibodies can be separated which are directed against factor VIII-related antigen. On the other hand, antibodies which are directed against factor VIII-related antigen have been reported to have varying degrees of cross reaction with factor VIII activity, possibly depending on the experimental conditions. This work has been extended recently by a paper (Ratnoff *et al.*, *Blood*, **47**, 657; 1976) which reports that antibody directed against factor VIII-related antigen does not inactivate the low molecular weight fragment of factor VIII even though it does react with native factor VIII.

Interpretations

A reasonable solution to some of the apparent differences in reported results is suggested in a paper (Kernoff, *Nature new Biol.*, **244**, 148; 1973) which shows that the antibody against factor VIII-related antigen will precipitate factor VIII but that the complex retains factor VIII activity. As a result, an apparent inactivation could be obtained if the supernatant solution was assayed after centrifugation. An interesting confirmation of this was also seen in immunoelectrophoresis experiments (Bird and Rizza, *Br. J. Haemat.*, **31**, 5; 1975) where the actual precipitin lines formed by factor VIII-related antigen and its antibody were shown to possess factor VIII activity. (The sample subjected to the immunoelectrophoresis contained both active factor VIII and antigen.) Clearly factor VIII and factor VIII-related antigen have determinants in common, or alternatively the two are bound together.

The bonding of active factor VIII subunits must rely on electrostatic attraction since ionic strength changes are sufficient to sever them. Chemical reduction has also been shown to disaggregate factor VIII and this might be due to cleavage of intramolecular disulphide bridges between different

parts of the same factor VIII subunit. When these are severed, different sections of the subunit could alter spatial configurations, and in that way geometrical changes would occur to a point where the electrostatic forces could no longer keep the subunits together. Bonds between the subunits of factor VIII-related antigen must be considerably stronger because more vigorous chemical reduction is required to disaggregate them. It would be anticipated that disulphide bonds are involved in some way but further conclusions are not possible at this stage.

Although there is considerable disagreement about the interpretation of the reported data on factor VIII, some useful conclusions can be drawn. Factor VIII-related antigen is shown to be a large molecule with a molecular weight of more than 10^6 . Active factor VIII can be separated from factor VIII-related antigen and to that extent they must be considered as separate entities. But they have intriguing similarities. They are difficult to separate unless factor VIII is cleaved into sub-

units and they have some antigenic determinants in common. The size of their subunits seems to be similar. Exercise and fear in the human subject bring about a proportionate increase in both. It is also important that the infusion of haemophilic plasma into a patient with von Willebrand's disease produces factor VIII activity.

These results are fascinating and most of them could possibly be explained on the basis of several hypotheses. In my opinion, however, the most reasonable hypothesis is that factor VIII and factor VIII-related antigen are separate molecular species with a basic structural similarity. This does not exclude the possibility that other species with that same basic structure are also present. It is interesting to speculate that such a substance, or even factor VIII-related antigen itself, could be the precursor of factor VIII. If this were the case, then treatment of haemophilia in the future might be based on converting a precursor into its active form rather than direct factor VIII replacement. □

Peptides in brain and intestine

from A. G. E. Pearse

IN 1931, while studying the distribution of acetylcholine, von Euler and Gaddum (*J. Physiol.*, **72**, 74) detected, in alcoholic extracts of equine brain and intestine, a smooth muscle stimulating and hypotensive agent which was later (Chang and Gaddum, *J. Physiol.*, **79**, 255; 1933) called substance P (for powder). Its identity as a peptide (protein) was soon established (von Euler, *Skand. Arch. Physiol.*, **73**, 142; 1936) but its isolation and characterisation had to wait for almost 40 years (Chang and Leeman, *J. biol. Chem.*, **245**, 4784; 1970; Chang, Leeman and Niall, *Nature*, **232**, 86; 1971).

The story of substance P can now be seen to provide nearly all the clues required for an understanding of the recent spate of reports of peptides common to brain and intestine. The latter region can more accurately be described as 'gastrointestinal tract and pancreas' and the peptides are regarded on the one hand as presumptive or proven neurotransmitters and, on the other, as presumptive or actual hormones.

A current list of the common peptides, with their original and new locations, is given in Table 1. The first two peptides in the list are primarily of neural origin. Demonstrated in brain by radio-immunoassay, with highest concentrations in the substantia nigra and hypo-

thalamus, substance P has now been localised, by immunocytochemical techniques, not only in the central nervous system (Hökfelt *et al.*, *Science*, **190**, 889; 1975), but also in some of the enterochromaffin (EC) cells of the gastrointestinal tract (Pearse and Polak, *Histochemistry*, **41**, 373; 1975) and bile ducts. This peptide (H-Arg-Pro-Lys-Pro-Gln-Gln-Phe-Phe-Gly-Leu-Met-NH₂) is probably widely distributed in primary sensory neurones and their processes, and in nerve terminals in many areas of the brain.

Somatostatin, named for its growth hormone release inhibiting properties, was isolated from ovine hypothalamus and characterised as a tetradecapeptide (H-Ala-Gly-Cys-Lys-Asn-Phe-Phe-Trp-Lys-Thr-Phe-Thr-Ser-Cys-OH) by Brazeau *et al.* (*Science*, **179**, 77; 1973). Later found to inhibit release of a much larger number of endocrine peptides, including insulin, glucagon and gastrin, somatostatin was detected by radio-immunoassay in the stomach and pancreas, in concentrations equal to those recorded in the hypothalamus (Arimura *et al.*, *Science*, **189**, 1007; 1975). It has been localised immunocytochemically in the neurones of the nucleus periventricularis of the hypothalamus, in nerve fibres in the neurohypophysis and

in some nerve fibres in the wall of the intestine (Hökfelt *et al.*, *Acta endocr. Suppl.*, **80**, 200; 1975).

Using similar immunocytochemical techniques several groups, independently, have now demonstrated somatostatin-like immunoreactivity in the D cells of the pancreatic islets and upper gastrointestinal tract. Consideration of the physical associations of the D cells with cells producing insulin, glucagon and gastrin leads to the supposition that the actions of the peripheral variety of this hormone are paracrine, directed that is to neighbouring cells and tissues, rather than truly endocrine. The last two peptides in Table 1 are products of two types of gastrointestinal endocrine cell belonging to the APUD series (Pearse, *Proc. R. Soc. Lond.*, **B170**, 71; 1968; *J. Histochem. Cytochem.*, **17**, 303; 1969). The name is an acronym based on their amine-handling qualities (amine content and/or amine precursor uptake and decarboxylation), but it stands for a larger spectrum of common cytochemical and ultrastructural qualities.

Gastrins, of whatever molecular species, are almost completely restricted to the G cells of the pyloric antrum of the stomach. Contrary to recorded opinion (Greider and McGuigan, *Diabetes*, **20**, 389; 1971), there is no gastrin in the adult endocrine pancreas.

Last year a gastrin-like peptide was demonstrated by immunoassay in extracts of human and animal brain (Vanderhaegen *et al.*, *Nature*, **257**, 604; 1975), and levels in the cerebral grey matter were as high as 200 ng per g of dry tissue. Since it eluted later than gastrin-17 on gel filtration, the new peptide must be regarded as closely related to, rather than identical with, gastrin.

Vasoactive intestinal peptide (VIP) is a 28-residue peptide originally isolated from porcine duodenum and shown to be structurally and biologically related to secretin and glucagon (Mutt and Said, *Eur. J. Biochem.*, **42**, 281; 1974). Present in the gastrointestinal tract, from oesophagus to rectum (Bloom and Bryant, *Gut*, **14**, 823; 1973), it was there shown to be produced by an APUD cell now tentatively identified as the H cell (Polak *et al.*, *Gut*, **15**, 720; 1974).

Two contemporaneous papers have appeared recently both recording the presence of VIP in neural tissues. Said and Rosenberg (*Science*, **192**, 907; 1976) have measured VIP immunoreactivity in three neuroblastoma cell lines in culture, finding high levels in all three and in acid alcohol extracts of the central nervous system and sympathetic ganglia. Their values in p mol per g (wet weight) of tissue from dogs, are higher in frontal lobe cortex (19) and hypothalamus (20) than in duodenum, ileum and colon. Bryant *et al.* (*Lancet*, **i**, 991; 1976) likewise report high levels of VIP

immunoreactivity in the cerebral cortex and hypothalamus of both man and pig, but in each case their average figures are lower than those recorded for the different regions of the gastrointestinal tract. In this second study a most important finding was that the bulk (94%) of VIP extractable from porcine and human brain elutes identically with, that is to say is virtually identical with, the pure intestinal peptide. Parallel immunocytochemical studies showed that in human and porcine intestine VIP is present not only in the endocrine VIP cells but also in some fine nerve fibres in the lamina propria, in the cell bodies of the myenteric plexus and in the adrenal medulla.

The two sides of the equation, neural and endocrine, are united by an essentially simple concept. First formulated some 10 years ago to explain the possession, by neurones and by endocrine cells producing polypeptide hormones, of a set of common cytochemical and ultrastructural characteristics, the APUD concept then postulated that the endocrine cells were derived from a common neuroectodermal ancestor. This was conceived as arising in the embryo from that transient but massive structure the neural crest, at one time considered as a fourth germ layer. Subsequent experimental work having shown that only seven of the 36 cells in the now amplified APUD series indeed arise from that structure, the concept has been extended to include, under the broad heading of neuroectoderm, not only the neural, tube, neural ridges and neural crest, but also the specialised ectoderm of the placodes (Pearse and Takor, *Clin. Endocr.*, Suppl. 5, 291; 1976).

It is now postulated that all peptide-hormone-producing cells are derivatives of specialised ectoderm and therefore, effectively, of cell lines derived from the epiblast and programmed for ultimate neuroendocrine function. Thus regarded, the cells of the APUD series constitute a third division of the nervous system, functioning as third-line effectors with activities slower in onset and of longer duration than those of the second-line effectors of the autonomic division. These, in turn, are of slower onset and longer duration than those of the somatic division.

If the concept is simple and concise, its implications are diverse and far reaching. They depend, to a large extent, on the nature of the several disciplines involved and these range from neurobiology (physiology, pharmacology and biochemistry), endocrinology and peptide chemistry, through conventional and comparative embryology to, if it yet exists, the molecular variety of that science.

Neurotransmitter peptides, many probably identical with individual endocrine peptides of the gut, and others related to the peptide products of exocrine (but still APUD) cells in the cutaneous glands of anurans (Erspermer

Table 1 Peptides common to intestine and brain

Peptide	"Original" location(s)	New location(s)
Substance P	Brain (cortex) Substantia nigra Dorsal root ganglia Peripheral nerves Substantia gelatinosa	Gastrointestinal EC cells
Somatostatin	Cerebral cortex N. periventricularis Neurohypophysis Pineal	Pancreatic islet D cells Gastrointestinal D cells
Gastrin	Gastric antrum (G cell) Duodenum	Cerebral cortex (grey matter)
VIP	Gastrointestinal tract especially duodenum, colon (H cell)	Cerebral cortex (grey matter) Hypothalamus

G, H and D, Wiesbaden classification of gastroenteropancreatic endocrine cells; EC, enterochromaffin; VIP, vasoactive intestinal peptide.

Table 2 Anuran and mammalian peptides with common amino acid sequences

Anuran peptide	Mammalian peptide
	Nervous system Endocrine system
Caerulein (<i>Hyla</i> sp.) (<i>Xenopus laevis</i>)	Gastrin Gastrin, CCK
Phyllocaerulein (<i>Phyllomedusa</i> sp.)	Gastrin
Physalaemic (<i>Physalaemus</i> sp.)	Substance P
Uperolein (<i>Uperoleia</i> sp.)	Substance P
Bombesin (<i>Bombina bombina</i>)	— Bombesin

CCK, cholecystokinin-pancreozymin.

and Melchiorri, *Pure appl. Chem.*, **35**, 463; 1973), will certainly be found widely distributed in the central and peripheral nervous systems. Their precise role in these situations can be envisaged as a matter of some complexity and fuller investigations of peptidergic neurotransmission will surely make it necessary at least to augment the older cholinergic and adrenergic notation.

The list of frog skin peptides with amino acid sequences common either to neural or endocrine peptides makes interesting reading (Table 2). Anuran cutaneous glands arise from the specialised ectoderm of the region adjacent to the embryonic neural plate and neural crest. They thus have a common origin with cells producing the brain and intestinal peptides which we have been considering and presumably they have evolved, in parallel with the nervous system, as a response to the (hostile) external environment.

A similar problem is posed by the existence of peptide products which are common to the nervous system, pituitary gland, gastrointestinal tract and placenta. Notwithstanding the nomenclature by custom attached to its principal layers, the cells of the endocrine placenta can be seen to arise from the neuroectodermal epiblast of the yolk sac, a layer which is continuous with the epiblast of the neural plate (Takor and Pearse, in preparation). Thus the many peptide hormones produced by the placenta can be linked with their counterparts in other regions.

The work of Steiner *et al.* (*Rec. Prog. Horm. Res.*, **25**, 207; 1969), on proinsulin

biosynthesis, provided protein chemists with a basis for several currently popular theories on the molecular evolution of peptide hormones. Steiner's own theory deriving insulin by way of a protopro-insulin from an ancestral intestinal protease, and Adelson's (*Nature*, **229**, 321; 1971) enterosecretory hypothesis deriving most peptide hormones from the entoderm of the primitive gut, seem to require reconsideration. Exact knowledge of structural correlations between brain and intestinal endocrine peptides is essential to proper understanding of their molecular evolution. Continued search for these peptides must be made in the earliest available vertebrates and in the invertebrate kingdom also.

For embryologists a clear challenge is posed by the close relationships now observed between neurotransmitter and endocrine peptides. In the hands of experimental embryologists the biological marker technique using avian chimaeras (Le Douarin, *Bull. biol. Franc. Belg.*, **103**, 435; 1969) has provided watertight evidence for the neural crest origin of a number of cells in the APUD series. Operative techniques of excision or exclusion (Andrew, *J. Embryol. exp. Morph.*, **31**, 589; 1974; Pictet *et al.*, *Science*, **191**, 191; 1976) have so far shown only that the gastrointestinal endocrine cells probably do not come from the definitive neural crest. Where they do come from is thus the problem that remains for the techniques of experimental embryology to answer.

Unquestionably the real future lies with the new science of molecular embryology.

This alone can provide an answer to the central question—how are individual cells in the embryonic epiblast programmed for neuroendocrine function and how, and at what stage, are the further series of instructions added which determine the ultimate endocrine function of each of the original cells? Already (Le Douarin, *Med. Biol.*, **52**, 281; 1974) we have learnt something of the timing and nature of the later stages of instruction but it is the problem of elucidating the earliest stages which provides the real challenge to experimental ingenuity. □

Cathodoluminescence of dislocations

from John Walker

DISLOCATIONS—misplaced planes of atoms which disturb the regular periodicity of otherwise perfect crystals—are important both theoretically and technically. They reduce a material's mechanical strength and affect its electrical properties; the quality and yield of electronic devices are inversely correlated with dislocation density. Hence dislocations have been extensively studied, and various techniques are used to observe them, including X-ray topography, electron microscopy and etch pit formation. A recent paper by Kiflawi and Lang (*Phil. Mag.*, **33**, 697–701; 1976) has demonstrated that a new weapon—cathodoluminescence—can be added to the technological armoury.

Cathodoluminescence is the release of light induced by electron bombardment, just as occurs in a television (cathode ray) tube. In earlier papers Lang and his colleagues showed that the growth history of a diamond crystal

could be studied using cathodoluminescence, and reported the existence of defects, suspected of being dislocations, which emitted a linearly-polarised blue luminescence. The most recent report demonstrates in a diamond a one to one correspondence between dislocations (as revealed by X-ray topography) and the blue-emitting lines (see Fig. 1); this proves beyond doubt that they are dislocations.

Features that appear in Fig. 1*b* but not Fig. 1*a* are dislocations which are too deep in the crystal to be observed in cathodoluminescence. Features in Fig. 1*a* that do not appear in Fig. 1*b*, although linear, are not dislocations. Their emission is not polarised and does not saturate in intensity with increased electron beam current, unlike the dislocation lines.

One problem with cathodoluminescence in the topographical examination of crystals is that it is limited to a layer within 100 μm of the surface by the penetration depth of the electrons. Consequently crystals need to be cut according to the surface to be studied. Furthermore, the crystal must be transparent to the emitted radiation. Diamond is ideal in this respect, because it transmits well into the ultraviolet, and can therefore be examined visually.

A great advantage of cathodoluminescence is its power of discrimination. Different types of feature can be distinguished easily. The possible variables are colour (blues, greens, yellows and pinks have been observed so far), polarisation of the light, and variation of luminescent intensity with electron beam current (some features saturate, others do not). No doubt cathodoluminescence will be used a great deal in the future for the study of dislocations and growth features in crystals. □

How closely did the continents fit together?

from A. Hallam

A FUNDAMENTAL tenet of plate tectonics, that amount of crust created at oceanic ridges must equal the amount subducted elsewhere, has recently been challenged in a stimulating paper by Owen (*Phil. Trans. R. Soc.*, **A281**, 223; 1976). He has argued that the components of Wegener's supercontinent Pangaea moved apart as a consequence of 20% expansion of the Earth's diameter since the early Jurassic, less than 200 million years ago. This iconoclastic view is very radical in terms of physical theory and is no more likely to be widely accepted than the similar view put forward some years ago by the Australian geologist Warren Carey. Bearing in mind the unfavourable reaction to Wegener's continental drift hypothesis earlier this century, and its subsequent history, it behoves earth scientists to preserve an open mind on such matters, but they have the right to demand compelling evidence in favour of earth expansion and a clear demonstration of the inadequacy of current global tectonic theory.

It can hardly be said that Owen is especially convincing on either count, which is not to say that he makes no telling points. The gist of his argument is geometric, that the celebrated Bullard fit of the Atlantic continents, and its successors such as the Smith and Hallam fit of the Gondwana continents, pose awkward problems of continental overlap and misfit which

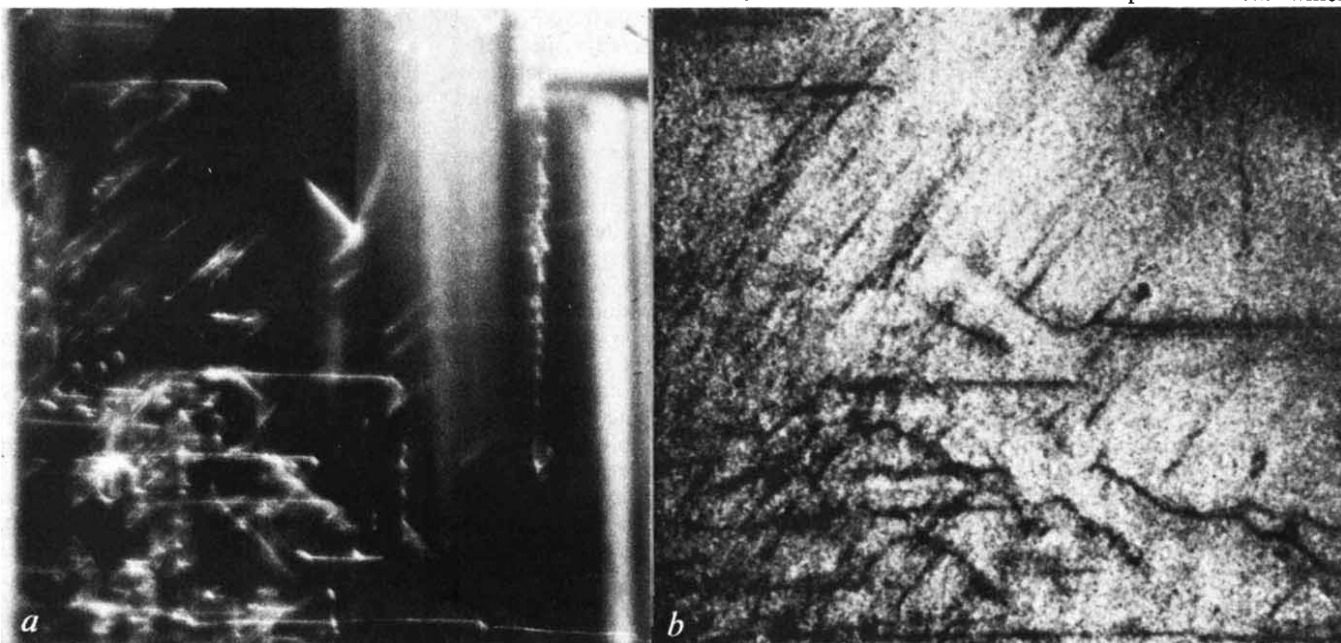


Fig. 1 Correspondence between cathodoluminescence images (a), and X-ray diffraction contrast images (b), of individual dislocations in a natural diamond.

have not been adequately explained away. For example, many geologists have been worried by the loss of much of Central America in the Bullard fit, despite the fact that extensive areas of old continental rocks occur there, and attempts to get round this problem by seemingly arbitrary *ad hoc* tectonic displacements and rotations, have lacked plausibility.

Likewise, if one adopts the Smith-Hallam fit, West Antarctica does not run naturally into its obvious geological continuation in Patagonia. More disturbing perhaps, a large gap is left west of Australia, which has led to the suggestion that the Wharton Basin in that region was ancient ocean, now disproved by the Deep Sea Drilling Project which has demonstrated that the basin is underlain by oceanic crust as young as elsewhere. On the other hand, fitting India against Australia, as others have done, leaves a corresponding gap in the western Indian Ocean.

Such problems can indeed be resolved, as Owen points out, by extremely rapid Earth expansion, but before leaping to such a drastic conclusion it is worth looking more closely at the original computer least squares fit pioneered by Bullard and his colleagues. This and subsequent fits were taken at the 500 fathoms (~1,000 m) contour for purely geometric reasons, because this gives the best fit and usually lies on the steepest part of the continental slope and is therefore well defined. It is only strictly valid if it coincides closely with the boundary of continental and oceanic crust, but geological and geophysical evidence has been accumulating that this is not the case.

Thus Talwani and Eldholm (*Bull. geol. Soc. Am.*, **83**, 3575; 1972) argue persuasively that the extensive Vöring Plateau off Norway, with an average depth of 2,000 m, is subsided and thinned continental crust. Similarly, Jansa *et al.* (*Geol. Surv. Canada Pap.* 74-30, 51; 1975) make a convincing case for a wide zone off the Nova Scotia, eastern Newfoundland and north west African shelves as subsided continent, and suggest that the 3,500 m contour is a geologically more realistic estimate of the true continental edge. Very similar evidence and arguments have been put forward for the region off-shore of Angola by Burollet and Byramjee (*Notes Mém. Comp. Fr. Pétroles*, no. 11, 71; 1974), leading to the same conclusion. An important implication is that a sector of subsided and attenuated continent well over 1,000 km wide in places must exist between Africa and America, and that the so-called Quiet Magnetic Zone is not entirely oceanic, as hitherto almost universally accepted by marine geophysicists.



A hundred years ago

VARIOUS sanitary measures (according to Dr Tholozan) have recently been adopted by the Turkish and Persian Governments with reference to the outbreak of plague, which commenced in Mesopotamia in the early part of the year. Since the beginning of March a sanitary cordon has been established on the north of the invaded territory, on the most frequented route of Kurdistan and Syria, between Tecrit and Kilfiri. On the south a quarantine of fifteen days is obligatory since April 1 on all vessels sailing on the Tigris and the Euphrates. It is at Kourna, at the confluence of these rivers. The ports of the Persian Gulf are protected by a quarantine which vessels from affected localities have to undergo at the island of Kezzer, formed by junction of the Chotel Aral and Karoun. Since April 10 all communications by land between Persia and Mesopotamia are subject to a quarantine of fifteen days. For three years, it may be added, all pilgrimages into the infected country, by Persian subjects, have been interdicted. from *Nature*, **14**, July 6, 218; 1876.

Burollet and Byramjee extend their zone of subsided continental crust round to the East African offshore zone and elsewhere, which has a significant bearing on the arguments recently put forward by Kent and Tarling (*Nature*, **261**, 304; 1976) against a fit of Madagascar snugly against Kenya and Tanzania. This they dismiss as implausible because of the known extent east of these mainland countries for hundreds of km of a thick sequence of late Palaeozoic and younger shallow marine and continental sediments. Kent and Tarling treat this as an argument for a more southerly former position of Madagascar, thereby dismissing without obvious justification palaeomagnetic evidence to the contrary, instead of appreciating that all that may be at fault is the geologically implausible tightness of fit if the 500 fathom contour is used. In this connection it is interesting to note that some new palaeomagnetic data just published supports the northerly fit for Madagascar (McElhinny and Embleton, *Earth planet. Sci. Lett.*, **31**, 101; 1976).

Much more research on continental margins around the world will have to be undertaken before an accurate new continental fit can be reliably attempted, but already it seems likely that most if not all of the apparent geological inconsistencies of the older fits will magically disappear, and the Tethys will not seem such an awkwardly eastward gaping ocean. □

Future of space science

from S. I. Rasool

A symposium on the Future of Science in Space was held as part of the 18th COSPAR meeting which took place in Philadelphia on June 7-18, 1976.

THE future of space science is becoming a matter of grave concern to the space scientist. The national space science budgets have considerably declined in their buying power and for the large pool of space experimenters in the Western world opportunities seem to be few. At the same time the exploratory phase in many disciplines is over. The days are long gone when a simple magnetometer, a geiger counter, or a TV camera flown anywhere in space or to a nearby planet would make an instant discovery. Although the potential for major discoveries about the Universe is still great in some of the fields (such as infrared, ultraviolet and gamma ray astronomy) the emphasis in space science is going to be more towards "why" than "what". This immediately implies more sophisticated and correlative experiments in space, large space telescopes and observatories to study the remote regions of the Universe and more detailed study of the planets with orbiters, landers and probes rather than with flybys.

What are the chances that space science will progress at the same vigorous pace as it has done over the past two decades? What should be the major thrusts and priorities? What are the national policies towards space science? These were some of the questions discussed in a one and one-half day symposium and panel discussion convened by COSPAR (International Committee on Space Research) during its recent meeting.

If the objective of the organisers was to get a unified view of things, the meeting was a disappointment. Six individuals from six different countries not only gave a variety of views but made six different kinds of presentations. They ranged all the way from topical and systematic descriptions of future thrusts in space research to a philosophical discourse on the art of extrapolation and on the meaning of utopia. A few themes did recur and are noteworthy: space research in the future will be more focused and will need more justification; with the advent of the Shuttle, the cost of doing science will, it is hoped, go down; space science cannot survive in isolation, it should be integrated with classical disciplines in science; high energy

astrophysics has a great future in space and the Space Telescope is the next logical step in optical astronomy; the scientific aspect of remote sensing of the earth will be emphasised more, while considerable thought should be given to studying the planets from earth orbiting telescopes.

Representatives from the European Space Agency (ESA) and NASA who joined the speakers for the panel discussion sounded optimistic about the future of space science in the total programme. ESA has a new science advisory structure to optimise the science input in the programme planning. NASA views space exploration closely tied to the biological imperative—the challenge of the unknown—and therefore a vigorous programme of space science and exploration has been and will be supported by the American public. A large Space Telescope, high energy observatories, probes to planets and a possible sample return from Mars may be the highlights of the US space science programme in the next decade. It appeared from the statements of the Soviet representatives that the Soviet emphasis on Venus will continue. In space astronomy, radio interferometry may be emphasised and in magnetospheric physics, active experiments to understand plasma interactions. An earth surface mineral resources survey was also mentioned by the Soviet scientists.

What was really accomplished at this Symposium is not clear and apparently not many people expected much to happen either: of the 600 or so participants at this year's COSPAR, the audience for this Symposium probably never reached the 100 mark. □

Trees in the tropics

from P. B. Tomlinson

The Fourth Cabot Symposium held at Harvard Forest, Harvard University, April 26–30 had as its subject Tropical Trees as Living Systems.

AN appreciation of the need for an understanding of tropical forests in terms of the individual diversity of tree species was the theme of the Symposium. The enormity of this task is indicated by the floristic diversity of woody plants in the tropics. In Malaysia, for example, M. E. D. Poore (*J. Ecol.*, **56**, 143–196; 1968) found in a survey of 23.0 ha, 2,773 tree-sized individuals with a trunk girth greater than 91 cm, representing 375 species, 139 genera and 52 families. To obtain an equivalent diversity in a temperate

flora one would have to map an area equal to a quarter of the United States. The systematic assessment of tropical tree floras is still incomplete, while most aspects of structure, growth, function, reproduction, and ecology remain uninvestigated. Generalisations about the biology of woody plants are still determined with temperate trees in mind; the more balanced view which might be obtained if tropical species were better understood has yet to be established. Consequently the uniqueness of this symposium was its examination of the tree as an individual, with a focus on many functional processes, and its attempt to deal mainly with tropical examples. The programme was so arranged that a gradual progression from a detailed consideration of construction and physiology led naturally to an appreciation of the tropical forest as a complex of interacting and highly diverse units. The result was an overview of our current understanding of aspects of the biology of tropical trees and the proceedings which are to be published should stimulate research on woody plants in the tropics.

Early sessions dealt with evolution, genetic diversity, and especially morphological patterns of organisation. The broad perspectives sought were indicated by the opening discussion of palaeobotanical evidence for the evolutionary origin of tropical trees and forests by J. Doyle (University of Michigan) which made the point that a better understanding of functional morphological relationships of leaf architecture would permit more conclusive palaeontological evaluation of theories of vegetative evolution in angiosperms. It was, therefore, appropriate that there should be a subsequent discussion of the adaptive significance of compound leaves by T. Givnish (Harvard University). This kind of interaction occurred throughout the meeting.

Tree architecture provided a framework for ample discussion, with emphasis on different kinds of trunk-branch axis differentiation in producing optimum patterns of leaf arrangement. The way in which the tree can often be regarded as a modular composition of equivalent units was emphasised by M.-F. Prévost (ORSTOM, Apidjoudmé, Ivory Coast). Of the many examples of crown construction presented, none was more distinctive than that of the genus *Araucaria*. In New Caledonia J. M. Veillon (ORSTOM, Noumea) showed that most species produced repeating crowns since the first generation of branches developed by the trunk is succeeded by two or more subsequent ones originating from late-developing branches, the successive crowns overlapping each other like a nest of coffee tables. The need for a

better appreciation of the complex hormonal control of branch expression was emphasised by A. Longman (Unit of Tree Biology, Institute of Terrestrial Ecology, Edinburgh) and G. Browning (University of Wales, Aberystwyth) but no better indication of the resulting regularity which is possible was found than in the quantitative analysis of *Terminalia* by J. B. Fisher (Fairchild Tropical Garden, Miami). This represents the first such precise analysis of a crown form familiar to tropical travellers and further demonstrated another common theme of the symposium, that quite simple methods and approaches can generate important new ideas. At the same time modern technology has its place, as was shown by R. Borchert (University of Kansas) whose computer simulation of shoot extension, based on known examples, suggests the existence of endogenous control of rhythms of shoot extension. Tropical trees are not necessarily subject to marked seasonal variation in climate and why shoot growth is usually not continuous is intriguing.

In the area of reproductive biology F. Ng (Forest Research Institute, Kepong) showed a greater diversity of seedling morphology in the tropics than the terminology devised for temperate trees can accommodate, with the obvious ecological implication of successful tree establishment as the basis for his study. The strategies that tropical trees have evolved in the evasion of seed predators were discussed by D. H. Janzen (University of Michigan). Predator pressure can be enormous and it was reassuring that J. Sarukhán (Universidad Nacional Autónoma de México) could find survivors enough to make demographic studies of continuing populations of forest trees.

The several speakers who attempted an analysis of the forest itself were unanimous in presenting it as a dynamic system. R. A. A. Oldeman (Mission ORSTOM, Ecuador) provided a provocative view of the forest as a population of trees whose developing architecture was the result of their individual ability to adapt to available energy levels through repeated cycles of growth. A contribution in which the data had been collected by University students (P. A. Ashton, University of Aberdeen) was particularly welcome since the tropical rain forest is an ideal training ground for future ecologists.

In all, 28 speakers from 13 countries participated. Such an overview is timely in view of the current rapid destruction of tropical forests, which was commented on repeatedly. At present the rate of increase in our understanding of tropical ecosystems is far exceeded by the rate of their destruction. Any corrective influence is appropriate. □

review article

Quasars and the cosmological distance scale

M. Rowan-Robinson*

There has been continuing controversy about the distances of the quasars since their discovery 13 years ago. Here one of the proponents of the view that at least some quasars are "local" explains why he now believes quasar redshifts are cosmological.

TWICE this century the cosmological distance scale has been the subject of famous debates. In 1920 Shapley and Curtis¹ debated whether the spiral nebulae formed part of the Milky Way system. Fifty-two years later, in December 1972, Arp and Bahcall² debated whether the redshifts of quasars are cosmological.

Both debates were inconclusive at the time. Shapley was correct in his estimate of the vast overall dimensions of our Galaxy: but on the question at issue he was wrong. In the second debate interesting and suggestive arguments (and not a few weak ones) were presented both for the "cosmological" and "local" interpretations of quasar redshifts, but neither proponent was persuaded to yield ground. A compromise solution, that two types of quasar exist, one type local, the other cosmological³, was unacceptable to both. Has the balance of the evidence shifted in the past three years? I believe that it has, and in this review I will try to explain why. For a more complete bibliography, the reader should consult Field *et al.*² and the more recent article by Burbidge⁴, whose conclusions are the opposite of those expressed here.

The 1972 Arp-Bahcall debate rested on 7 main kinds of argument; (1) the redshift-distance relationship for normal galaxies, (2) continuity between quasars and other types of active and compact galaxy, (3) quasars in the directions of galaxies and clusters of galaxies with the same redshift as the quasar, (4) quasars in the same direction as bright galaxies of much lower redshift, (5) luminous filaments connecting galaxies and quasars with very different redshifts, (6) the overall distribution of quasars on the sky, (7) non-velocity redshifts in normal galaxies.

While the anomalies (4)–(7) have been neither impressively augmented in the interim, nor decisively eliminated, the evidence under (1) and (2) has strengthened considerably.

The redshift-distance relationship for normal galaxies

In a classic series of papers Sandage and Tammann^{5–11} have put the redshift-distance relationship discovered by Hubble and Lundmark on to a solid basis, and have removed the possibility of any strong deviation from this linear behaviour within 200 Mpc.

In the first two papers^{5,6}, galaxies of the Local Group and of the nearby M81 group (3.25 Mpc away), for which distances can be established from Cepheid variable stars (assuming the known distance of the Hyades cluster), are used to calibrate two methods that can be used for more distant galaxies: the optical size of the largest H II regions in galaxies and the luminosity of the brightest stars in a galaxy. In both cases the quantities to be used as distance indicators depend on the luminosity and

type of galaxy concerned, and this dependence has to be obtained from these nearest galaxies. In the third paper⁷ those two methods are used to determine the distance of the M101 group of galaxies (7.2 Mpc), important because it provides the nearest example of an Sc galaxy of luminosity class I. van den Bergh¹² found that the appearance (surface brightness, degree of development) of spiral arms is correlated with the luminosity of the galaxy, and may be used to provide a luminosity classification of spiral galaxies. In the fourth paper⁸ the same two methods are used to get the distances of 39 Sc galaxies with distances in the range 3–20 Mpc, and hence to calibrate the van den Bergh luminosity classes. In the fifth paper⁹ distances to groups of galaxies within 30 Mpc are determined both by the H II region method and by Sc luminosity classes. The latter method is also used for more distant galaxies (to 50 Mpc). A convincing redshift-distance relation is obtained, the constant of proportionality being $H_0 = 57 \pm 3 \text{ km s}^{-1} \text{ Mpc}^{-1}$ (the Hubble constant) and this linear relationship is "not only as isotropic as it can presently be determined ($3\sigma [\Delta H/H] \approx 30\%$), but the flow is also as quiet as we can measure it". In fact Sandage and Tammann allow only $\sigma(\Delta v) \lesssim 50 \text{ km s}^{-1}$ as "a generous limit for the mean random motion" (of galaxies about the Hubble flow). In the sixth paper¹⁰ the authors use a sample of remote Sc I galaxies (out to 200 Mpc) to determine $H_0 = 55 \pm 6$. Finally Sandage¹¹ uses a further remote sample of galaxies, from the Southern Hemisphere, to check the isotropy of the Hubble flow.

Sandage¹³ had earlier shown, continuing the programme of Humason, Mayall and Sandage¹⁴, that the brightest galaxies in rich clusters show a good correlation between magnitude and redshift, consistent with the Hubble relation together with the assumption that the luminosity of the brightest galaxies in clusters are all the same. This pushes the frontier to which the Hubble law has been tested to almost 3,000 Mpc.

Sandage and Tammann have concentrated on the four distance methods: Cepheids, H II regions, bright stars and Sc luminosity classes. van den Bergh¹⁵ reviews distance estimates to the nearby Magellanic clouds by 11 different methods and shows that the methods broadly agree with one another. He also reviews the results of 12 methods of determining H_0 and arrives at a weighted mean of 93 ± 7 . Most of these methods have, however, large uncertainties and the Sandage and Tammann value of 55 is within 2.6σ of all of them. It is also not clear that most of these methods have been calibrated with the same care as the Sandage and Tammann programme, especially for correlations of the chosen indicator with galaxy type and luminosity. The most important alternative method is one based on the expansion rate of supernovae¹⁶, which is completely independent of the whole elaborate distance ladder built up from the Hyades cluster. This yields $H_0 = 60 \pm 15$,

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an impressive and rather remarkable confirmation of the Sandage and Tammann distance scale.

Dissenting points of view

Three of the main dissenting points of view focus on the questions of calibration, the Local Supercluster, and the so-called Rubin-Ford anomaly. The sceptic might also ask a fourth question: will the Hubble constant be reduced further?

Two criticisms have been directed at Sandage and Tammann's calibration of their distance scale. Madore¹⁷ has argued that additional corrections need to be applied to the determination of distances using Cepheid variables, which would have the effect of increasing H_0 , and Denmat *et al.*¹⁸ claim that with the Sandage and Tammann Sc luminosity class calibration, H_0 is different when determined by galaxies in different classes. If van den Bergh's original calibration is used, however, this difference disappears: an embarrassing side effect is then that H_0 decreases with distance. A dependence of the Hubble constant on distance had earlier been claimed by de Vaucouleurs¹⁹.

Because of the rather small number of galaxies available for the calibration of each luminosity class, it is rather difficult to decide between an incorrect calibration of the relative luminosity of the different classes, and a dependence of H_0 on distance. The linearity of the magnitude-redshift relationship for brightest cluster galaxies¹³ shows that such a dependence can only occur for distances nearer than the Virgo cluster, which fits well on to the relation for other clusters. An important argument against a dependence of H_0 on distance is therefore that the value of H_0 determined from the Virgo cluster alone⁸ agrees well with that determined from all nearby galaxies.

The second issue is the long standing one of the local supercluster. de Vaucouleurs²⁰ has argued that the prominent band of galaxies across the north Galactic pole and passing through the Virgo cluster constitutes a disk-like "supercluster" of galaxies with the centre at Virgo and ourselves at one edge. The Sandage and Tammann programme deals a severe blow to this hypothesis, since they find no deviation from the linear Hubble flow correlated with direction along the supergalactic plane, or towards and away from Virgo. Jones²¹ has argued that the local galaxy distribution can be understood in terms of Virgo being a rich cluster of normal dimensions (diameter ~ 4 Mpc, instead of ~ 40 Mpc for the supercluster). de Vaucouleurs has, however, reiterated his case for the existence of the supercluster, using evidence from nearby galaxies²², hydrogen clouds²³, groups of galaxies²⁴, and clouds of galaxies²⁵. In each case the number of objects seems rather small for a significant determination of distribution on the sky. A further problem is the strong effect of galactic obscuration below galactic latitude $b=40^\circ$, so that only about one third of the sky can be studied in a relatively unobscured direction.

Third, cosmologists have been perplexed by the Rubin-Ford anomaly. Rubin, Ford and Rubin²⁶ studied a sample of 50 ScI galaxies with $14 \lesssim m_{pg} \lesssim 15$, and $4,000 \lesssim v \lesssim 7,500$ km s⁻¹. They found different mean velocities for two hemispheres of the sky corresponding to a velocity of 700 km s⁻¹ for the Solar System relative to these galaxies. Since our velocity relative to the microwave background is $\lesssim 300$ km s⁻¹ (ref. 27), a paradox of Michelson-Morley proportions seemed to be emerging. Jaakola *et al.*²⁸ claim a difference in the redshift distribution for compact galaxies with absorption spectra, over the two Rubin-Ford hemispheres, but only at the 2 σ level.

Sandage and Tammann⁹ find no evidence for a difference in H_0 between the two hemispheres when velocity-limited samples are studied. They conclude that the effect is due to the bias introduced by a magnitude-limited sample of a population in which there is an appreciable spread in the luminosity. Hartwick²⁹ discusses the contribution of interstellar absorption to such an effect.

Finally, the value of H_0 first given by Hubble³⁰ was 500, since when the accepted value has been steadily reduced.

Have we reached the end of the road, or will there be yet another downward revision in a decade's time? Tammann³¹ explains why the corrections have always tended to be downwards: "one is basically reluctant to accept the existence of objects which are much brighter and much larger than those one has previously experienced in the immediate neighbourhood". The value $H_0 = 55$ leads to an expansion time for the Universe, if the redshift is interpreted as due to recession, of 1.9×10^{10} yr, compared with the age of our Galaxy of 1×10^{10} – 1.5×10^{10} yr, fitting in well with the idea of a 'big bang' universe in which galaxies form early on.

(There is, unfortunately, no direct evidence for interpreting the redshift as due to recession. The success of the big bang picture in explaining the microwave background, the primordial abundances of He and D, and the age of our Galaxy, constitute powerful indirect evidence. Most of the discussion of this article is independent of the interpretation of the redshift.)

At present it is hard to imagine H_0 lying outside the range 30–100, but there are two arguments which might eventually force a lower value. If the local supercluster could be shown to be a real object 40 Mpc in size, then for it not to be the largest cluster in the Universe, H_0 might have to be as low as 10. But the recession velocities of local supercluster galaxies would then be anomalously large. Second, the revision of stellar evolution theory needed to explain the low solar neutrino flux could substantially increase the life of stars on the main sequence, and hence the age of the oldest stars in the galaxy.

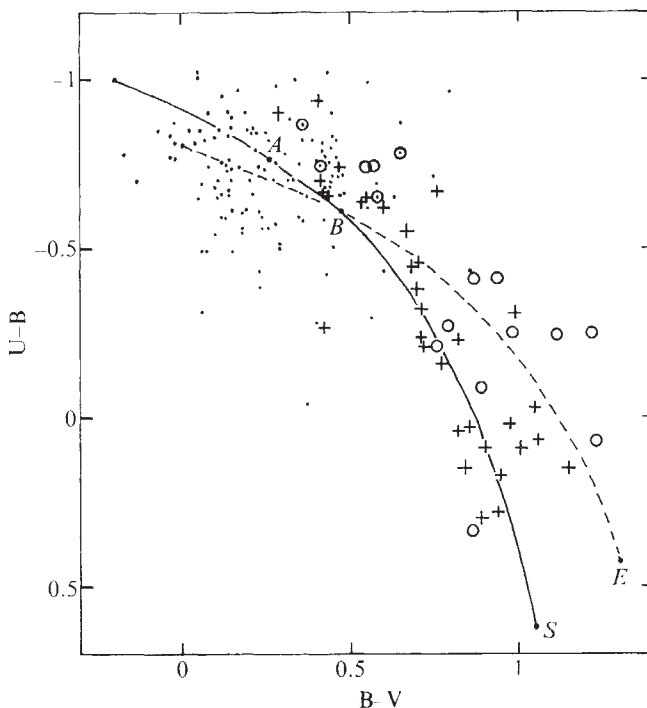


Fig. 1 Colour-colour (U-B against B-V) diagram for quasar (●, ref. 40), N galaxies (○, ref. 37) and Seyfert galaxies (+, refs 38, 39), together with mixture lines calculated by combining the light of a normal galaxy with that of a quasistellar nucleus, in different proportions (spiral at $z = 0$, $B-V = 1.05$, $U-B = 0.62$; solid curve (ref. 38); elliptical at $z = 0.1$, $B-V = 1.3$, $U-B = 0.53$; broken curve (ref. 37)). The circled dots at (0.36, -0.87), (0.58, -0.65), (0.41, -0.74), (0.64, -0.78) denote B46, B264, B340 and Ton 256, respectively, which have been classified as quasars, but which are fuzzy. The points labelled A and B on the spiral and elliptical loci, respectively, denote the points at which the visual light from the quasistellar core equals that from the galaxy. No Seyferts are found to the left of A and no N galaxies are found to the left of B.

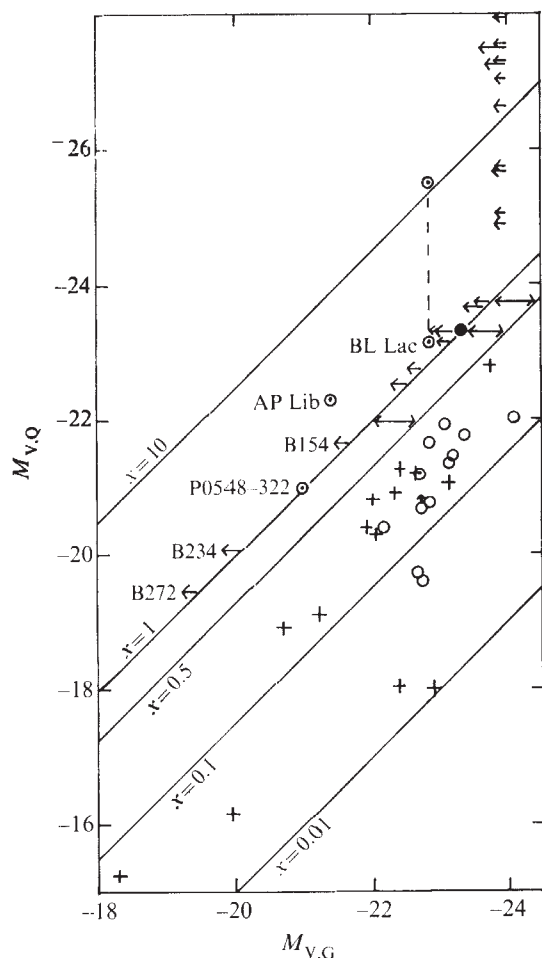


Fig. 2 Comparison of the absolute visual magnitudes of the quasistellar ($M_{v,q}$) and galaxy ($M_{v,g}$) components in Seyferts (+, refs 38, 39, 42), N galaxies (○, ref. 37), BL Lac objects (○, refs 43–46) and optically selected QSOs (Ton 256 as filled dot, rest as arrows, refs 40, 47, 48). The redshifts of QSOs have been assumed to be cosmological, and H_0 has been taken as 50. Lines of constant $x = \rho_{oc}/\rho_{og}$, the ratio of the visual light from the core to that from the surrounding galaxy, are shown. B46, B264, B340, which are fuzzy, are shown as having $0.5 < x < 1$, and the rest of the QSOs are shown with $x \geq 1$ or $M_{v,g} > -24$, the highest luminosity attained by galaxies.

Continuity between quasars and galaxies

The similarities between quasars and the nuclei of N galaxies and Seyferts have been commented on by many authors^{32–36}. Sandage³⁷ showed that the colours of N galaxies as a function of aperture could be understood in terms of underluminous QSOs in the nuclei of normal giant elliptical galaxies. Weedman³⁸ and Penston *et al.*³⁹ have shown a similar relationship between Seyfert nuclei and spirals. Fig. 1 shows the colour–colour diagram for quasars, N galaxies and Seyferts, together with the loci obtained by adding quasars of different luminosity to normal elliptical and spiral galaxies.

Kristian⁴¹ showed that amongst low redshift quasars, those with lower optical luminosity (assuming the redshift to be cosmological) tended to be fuzzy, while those of higher optical luminosity appeared stellar, which would be consistent with the idea of an underlying giant elliptical galaxy. Figure 2 compares the optical luminosities of the quasistellar (ρ_{oc}) and galactic (ρ_{og}) components in quasars, N galaxies and Seyferts. We suppose that quasars which have been described as fuzzy lie in the band $x = \rho_{oc}/\rho_{og} \sim 0.5$ –1. If $x \geq 1$ the object appears entirely stellar and is called a quasar: if $x \lesssim 0.5$ the object is sufficiently fuzzy to be called an N galaxy or Seyfert. In between there is a domain of uncertainty where objects are classified as quasars or N galaxies by different observers (for example, Ton 256, B264).

The BL Lac objects

The most interesting development of the past few years has been the discovery of the BL Lac objects. These are strong variable radio sources identified with QSOs of neutral colour, in which the spectra show no emission lines⁴². This seems to be due to a steep decline with increasing frequency of the optical continuum (also responsible for the neutral colours), with a consequent deficiency of ultraviolet photons to excite the lines⁴³. They are therefore simply quasars without an ultraviolet excess.

Table 1 lists the properties of the BL Lac objects identified to date. Oke and Gunn⁴³ succeeded in measuring the spectrum of the galaxy underlying BL Lac, with a redshift of $z = 0.07$. Baldwin *et al.*⁵⁰, however, failed to find any features in their spectrum of BL Lac. Thuan *et al.*⁴⁴ confirmed the redshift of $z = 0.070 \pm 0.005$, on the basis of several normal galaxy lines and bands. It would be clearly desirable to have this result confirmed by independent observers. Other BL Lac objects which have had redshifts determined are P0735+178⁵¹, P0548–322⁴⁵, AP Lib⁴⁶ and B2 1101+38⁵². The separation into galactic and quasistellar components has been performed for BL Lac⁴³, P0548–322⁴⁵ and AP Lib⁴⁶, and they are included in Fig. 2. For AP Lib, $x \sim 3$, and for BL Lac, $x \sim 10$ when observed by Oke and Gunn⁴³, so these objects are firmly into the quasar band in Fig. 2. The identification of underlying galaxies in these objects is therefore one of the strongest pieces of evidence that quasars occur in galaxies. The high optical luminosity achieved by BL Lac at maximum removes any rationale for treating the quasars with flat spectrum, compact radio sources as a local population with properties similar to the low luminosity sources seen in Seyfert nuclei³. This type of activity is clear possible over a wide range of luminosity, up to the range seen in quasars, and no advantage is gained by the “local” hypothesis.

The strong variability of some quasars and BL Lac objects suggests that they may sometimes be found in a quiet phase, with $x \lesssim 1/2$, that is, becoming N galaxies. Spectra and photographs taken at these phases should show evidence of an underlying galaxy. Figure 2 shows, however, that the notion that QSOs are only to be found in giant ellipticals should be dropped. B154 and B234 show no fuzz even though they are respectively 1.6 and 3.2 magnitudes fainter than the mean for brightness cluster ellipticals. These QSOs can be occurring only in galaxies of modest optical luminosity.

Finally the case of 3C48 should be mentioned. Wampler *et al.*⁷⁴ succeeded in detecting emission lines from hot gas associated with the nebulosity surrounding this quasar. The lines are narrower, and have a slightly larger redshift, than those of the central quasar, thus ruling out the gravitational redshift. The high optical luminosity of 3C48 ($M_v = -25.5$) makes it unsurprising that an underlying galaxy was not detected.

Associations on the sky

Arp² has emphasised how apparent associations on the sky have an important role in establishing the conventional distance scale for galaxies. Groups and clusters of galaxies seen in a particular direction on the sky are normally assumed to be at the same distance.

Several quasars of low redshift have been found to be “near” galaxies and groups of galaxies of the same redshift (Table 2a), presumably establishing the cosmological nature of the redshift in these quasars (but this is disputed by Burbidge and O’Dell⁵⁹). It is unfortunate that all the clearcut cases of this type are quasars with extended double radio sources, for which the continuity of the radio dimensions with those in radio galaxies and the fall off of angular size with redshift already support the cosmological interpretation.

The best case involving a compact radio-source quasar is 4C11.50B = 1548+115a⁶⁰, but here two quasars are found near a group of galaxies^{91,92}, one with the same redshift as the group⁸⁰, one much larger, and it is unclear which is responsible

Table 1 Properties of BL Lac type objects

Name	Redshift	B	B-V	S(5GHz) (Jy)	$\alpha(5\text{GHz})$ [S(v) αv^a]	Refs for data	$M_{V,Q}$	$M_{V,C}$	$\log$ $P(5\text{GHz})$ (W Hz ⁻¹)
P0048-097 = OB-080		17.08	0.48	2.0	0.45	53, 54			
0219 + 42 = 3C66A		15.71	0.50	0.91	0.3	55, 56			
AO 0235+164		16.2-18.2	1.3	1.4-2.8		57			
0300+47 = 4C47.08 = OE400		18(pg)		2.4	0.0	58, 54			
P0537-441		12.6-16.9	0.5	5.0	0.7	59-61			
P0548-322	0.042	15.6	0.79	0.23	-0.52	45	-21.0	-21.0	24.2
P0735+178 = OI158 = VRO 17.07.02	0.424	14.5-16.7	0.52	2.1	-0.04	51, 60	-26.3		27.2
MA0829+04		15-16.9		0.95	0.15	62, 60			
0851+20 = OJ287 = VRO20.08.01		12.35-16.4	0.4	3.5	0.5 $\pm$ 0.2	63, 49, 54			
B20912+29		15.1-17.0	0.34	0.24	-0.35	62, 53, 54			
UT0954+556 = OK 591 = 4C55.17				3.53	-0.28	64			
UT1020-103 = OL-133 =		16(V)		(1,415 MHz)	0.7	65, 66			
MSH 10-107				(1,415 MHz)	0.19				
1057+10.0		17.60	0.38	(2,700 MHz)		53, 67			
B2 1101+38	0.0308	13.1(pg)		0.54		52, 54	(-23.2)		24.3
B2 1147+24.5 = OM280		16.28-16.61	0.5	0.59	0.13	53, 68			
B2 1215+30 = ON325		13.9-16.0	0.5	0.33	-0.2 $\pm$ 0.2	63, 49, 54			
B2 1219+28 = W Com = ON235		13.2-17.2	0.5	1.2	0.25	69, 49, 54			
1514-24 = AP Lib = OR-225	0.0486	14.0-16.4	0.6	2.2	0.0	46, 63, 49, 54	-22.3	-21.4	25.3
1538+149 = OR165 = 4C14.60		14.4-17.8	0.5	2.0	0.2	62, 60, 53			
B2 1652+39 = Mark 501	0.0337	14.60	0.72	2.0(408 MHz)	70		(-22.7)		(25.0)
1727+50 = I Zw187 = OT546		14.8-16.9	0.7	0.15	-0.4	63, 71, 54			
1749+09 = OT081 = LC 09.57		18(V)		1.83	0.1	72, 68			
2200+42 = BL Lac = OY401 =	0.070	12.6-16.6	1.0	7.0	0.75	43, 44, 63, 54	-23.15	-22.86	26.2
VRO 42.22.01						73	to -25.5		
2207+02		19(pg)							
2254+07 = OY091		15.7-17.3		1.0	0.15 $\pm$ 0.1	62, 60			
P2335+03		18(pg)		0.61	0.64	73, 68			

The spectral indices are taken from ref. 62.

The luminosities given in the last 3 columns are calculated assuming $H_0 = 50$.

Note added in proof: Twelve further possible BL Lac objects are given by Kinman¹²⁵. E. M. Burbidge *et al.*¹²⁶ have measured the absorption time redshift of AO 0235+164 as 0.5240, with a possible second system corresponding to $z = 0.85$.

for the associated extended double radio source. Argue *et al.*⁹⁰ propose that the double source, as well as the compact source, is associated with the smaller redshift quasar, but the centroids of the two emission regions are accurately aligned with the high redshift quasar. Clearly it is important to find unambiguous examples of low redshift, compact radio-source quasars in groups or clusters of galaxies.

Many examples have also been given of quasars "near" comparatively nearby galaxies, of much lower redshift than the quasar (Table 2b). Arp and G. R. Burbidge have been the strongest proponents of the arguments that these associations are real and that the redshifts of many or all quasars include a substantial intrinsic component. *Post hoc* calculations of the probability of these associations have been adduced as support for this interpretation^{2,93}, but all such calculations are fallacious from a philosophical point of view. Evidence that such associations are real must be of a statistical nature. A chosen large sample of bright (peculiar?) galaxies must be selected in advance, together with a comparable random set of positions on the sky as a control. Regions of the same size should be

searched round each position in the two samples for quasars, and the redshifts determined. In this way clearcut evidence that quasars are to be found around bright galaxies can be obtained.

The number of close associations found to date is not especially remarkable. The number of quasars on the whole sky, N , brighter than magnitude B_Q and within $2'$ of a galaxy brighter than m_G , is roughly

$$\log_{10} N \approx 2.9 + 0.75(B_Q - 20) + 0.6(m_G - 15.7)$$

using Sandage and Luyten's⁹⁴ $N-m$ relation for QSOs and the fact that there are on average about 1.6 galaxies per square degree in the Zwicky catalogue⁹⁵. At present perhaps 1-2% of the sky has been searched for associations between quasar and Zwicky galaxies, giving a prediction of 8-16 such associations as compared with the 12 given in Table 2.

Two cases of pairs of quasars very close together (Ton 155, 156, $35''$ apart⁹⁶; 4C11.50a,b, $5''$ apart^{91,92}) have been given. It is interesting that Stockton⁹⁰ has found a galaxy near 4C11.50

Table 2 Associations of quasars with galaxies

Year	a, Associations with galaxies of the same redshift	b, Associations with galaxies of different redshift (within $2'$ arc)
1969	B264 in cluster with $z = 0.095$ (75)*	1108+285 1.1' from NGC3561 (81)
1970		Mark205 42'' from NGC4319 (82)
1971	P2251+11 in cluster with $z = 0.323$ (76)	3C232 1.9' from NGC3067 (83)
	Ton256 in cluster with $z = 0.131$ (76)	PHL226 0.8' from IC1746 (83)
1972	3C323.1 in cluster with $z = 0.264$ (77)	3C455 23'' from NGC7413 (84)
		P1021 -0.0 1.9' from Zwicky galaxy (89)
		P2020 -37.0 15' from galaxy (86)
1973	RN8 (near 3C61.1) in cluster with $z = 0.184$	P0038 -0.1.9 1.2' from Zwicky galaxy (61)
	4C37.43 associated with galaxy $z = 0.370$ (79)	P0823 +03.3 1.7' from Zwicky galaxy (61)
1974	4C11.50a near galaxy with $z = 0.436$ (80)	
1975		Quasars 76'' from IC1417, 95'' from NGC5682 (87)
		QSO 58'' from IC2402 (88)

*Numbers in parentheses are ref. numbers.

with the same redshift as the low redshift quasar. Many more examples would have to be found (and again, in a well-defined search programme) to constitute evidence that these are real associations.

The situation on luminous filaments connecting galaxies with very different redshifts^{2,97-101} remains inconclusive. Walker *et al.*¹⁰² find no clear luminous connection between NGC4319 and Markarian 205, or between the members of Seyfert's Sextet. They confirm the reality of the arm linking NGC7603 to its companion, but find no evidence of interaction.

Other evidence

Among the additional arguments cited in favour of the cosmological interpretation of quasar redshifts are (1) the decline with redshift of the maximum separation found in extended double radio sources in quasars¹⁰³⁻¹⁰⁷ (2) H I absorption in quasars which may be due to an intervening gas cloud^{108,109} (3) interpretation of some ultraviolet absorption lines as due to molecular hydrogen between us and large redshift quasars^{110,111} (4) various claims of correlations of optical and radio brightness with redshift (of somewhat doubtful significance in view of the wide range of quasar radio and optical luminosities).

Supporting the "local" interpretation are (1) periodicities in the redshift distribution of quasars (and radio galaxies)¹¹⁵⁻¹¹⁷ (2) excess redshifts in normal galaxies¹¹⁸⁻¹²² (3) rapid radio variations requiring ultrarelativistic velocities¹²³ (such motions are anyway required in some radio galaxies and to understand multiple absorption redshift in quasar spectra).

None of these arguments seems really decisive either way.

Conclusion

The establishment of the redshift-distance relationship for galaxies on what seems to be an impregnable basis, to the extent that any "intrinsic" redshift effect cannot exceed 50 km s⁻¹, and the evidence that QSOs occur in galaxies and with luminosities at least ten times that of the galaxy, removes any doubt from the present author's mind that the redshifts of quasars are cosmological. Certainly there no longer seems to be any strong reason for maintaining that quasars with compact radio sources are local^{3,124}. While efforts to detect galaxies believed to underlie quasars, and to find associated groups of galaxies, should clearly continue, supporters of the "local" hypothesis have to meet the challenge of pursuing a well defined, unbiased, controlled programme to demonstrate statistically the association of quasars with relatively nearby galaxies. On the present evidence I believe that such a programme will fail.

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articles

The Karari Industry: Early Pleistocene archaeological evidence from the terrain east of Lake Turkana, Kenya

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Information concerning the technology, land-use patterns and activities of early hominids is being recovered from the Upper Member of the Koobi Fora Formation. The term Karari Industry is proposed for a distinctive local series of artefact assemblages that date to the period between 1.2 and 1.6 Myr BP.

THE area east of Lake Turkana (formerly Rudolf) is now well known for the large series of hominid and other fossils that Leakey *et al.* have recovered there¹⁻⁸. Researches in the area since 1970 have also yielded an abundance of archaeological traces that relate to the activities and behaviour of early hominids. Two series of archaeological occurrences can be recognised. An older series which occurs in the top of the Lower Member of the Koobi Fora Formation, chiefly in the KBS Tuff complex, has been the subject of preliminary reports⁷⁻⁹. The artefacts from these sites can be tentatively termed the KBS Industry. A younger, contrasting series has subsequently been discovered in the Upper Member. This is described here for the first time, and we propose that it be designated the Karari Industry.

Provenance and stratigraphy

Sites pertaining to the newly discovered archaeological entity occur in great profusion along a low eroded ridge that has come to be known to our research group as the

Karari Escarpment (Figs 1 and 2) and we have used the association with this topographic feature in naming the industry. Samples of the industry, however, have also been recovered from the dip slope to the east of the Karari escarpment, and from the Ileret area. The locations of known sites are marked on Figs 1 and 2, but other areas remain to be explored and it is probable that the distribution will eventually prove wider than that shown.

All sites are designated in accordance with the standard African site enumeration system¹⁰. The letters identify a series of geographic rectangles bounded by lines of latitude and longitude at one quarter degree intervals. Thus FwJi denotes the square 4°15'–30'N and 36°0'–15'E while FxJj refers to the square 4°0'–15'N and 36°15'–30'E. The digits that follow are catalogue numbers that reflect the sequence of registration.

Table 1 summarises the stratigraphic relationships of the sites to each other and to the named units designated by Bowen and Vondra¹¹. Along the Karari escarpment and on the dip slope to the east, the sites are stratified in fluvial deposits between the KBS Tuff complex and the Karari Tuff. They are particularly abundant in the strata associated with the Okote Tuff complex (formerly the BBS Tuff complex)¹¹. They occur scattered through a cumulative thickness of some 50 m of sediments in the Karari area. In the Ileret area the sites occur in low energy river floodplain

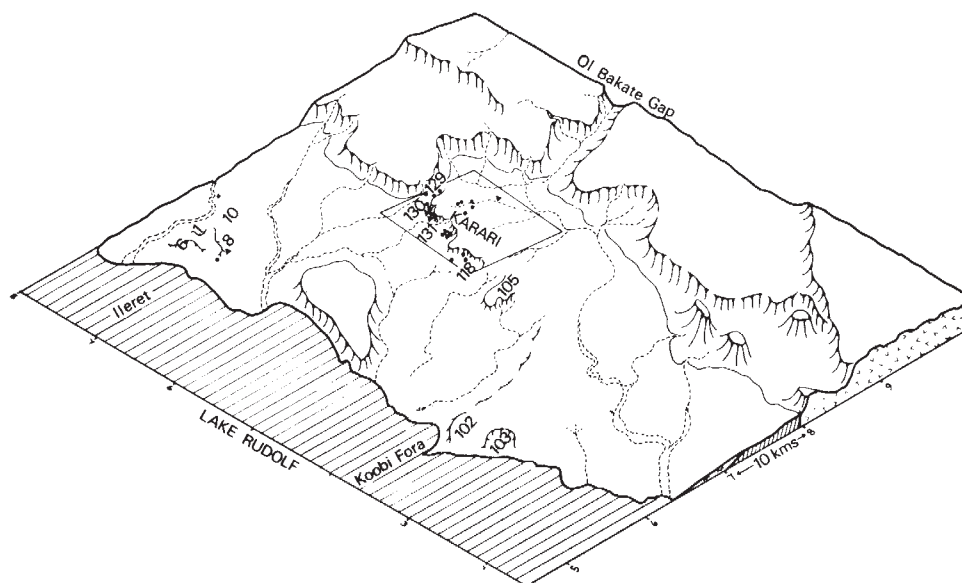


Fig. 1 Isometric block diagram showing the position of the Karari and Ileret sites in relation to present-day physiography. Numbers 1-131 refer to the expedition area numbers; rectangle designates the area shown in Fig. 2.

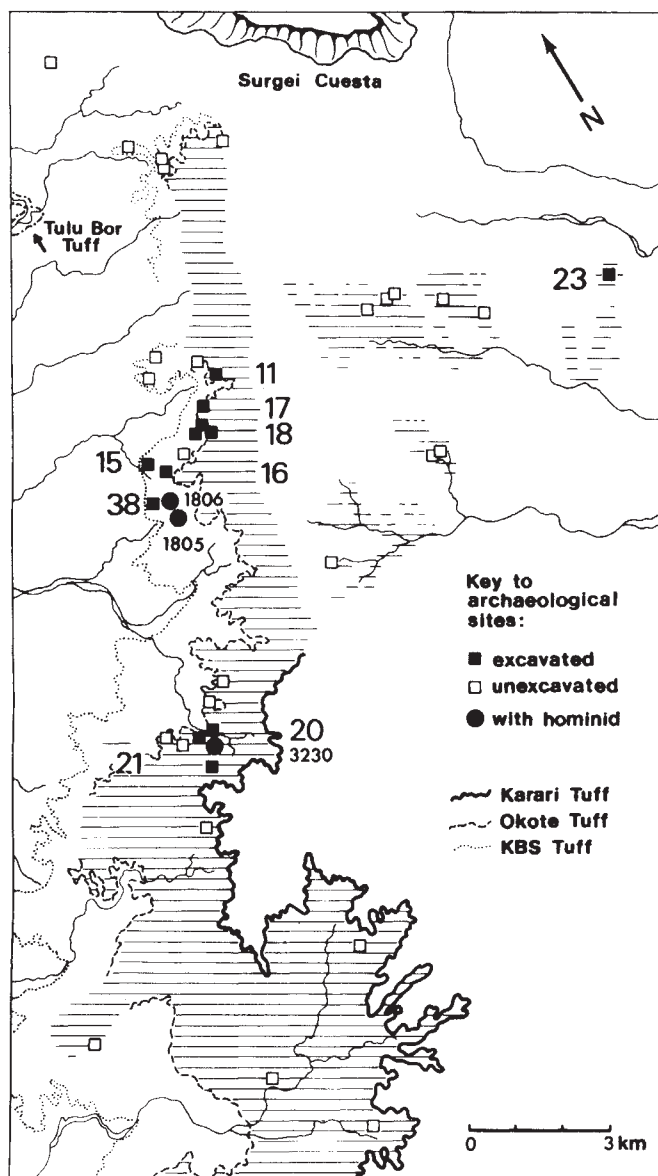


Fig. 2 Plan showing the location of sites along the Karari Escarpment. The geology is based on data from I. Findlater. All two-digit numbers refer to site catalogue numbers within grid square FxJj. Four digit numbers are Kenya National Museum (KNM-ER) catalogue numbers for hominid fossils. Hatching shows outcrops of beds between the KBS and Karari Tuffs.

and delta floodplain deposits between the tuffs previously referred to as the Ileret Lower and Middle Tuffs¹². These are correlated with the Okote Tuff complex. The Ileret sequence is capped by the Chari Tuff, which is believed to correlate with the Karari Tuff, and thus the entire series of sites treated in this report can be said to derive from the stratigraphic interval between the KBS Tuff complex and the Chari-Karari Tuffs. Lake margin sediments that correlate with this interval also crop out in the vicinity of the Koobi Fora spit, but archaeological materials have not been recovered from these exposures.

In biostratigraphic terms, the interval from which the samples of the Karari Industry derives coincides largely with Maglio's *Loxodonta africana* zone¹³.

Dating evidence

Table 2 shows the series of published potassium-argon determinations by Fitch and Miller that have a direct bearing on estimation of the age of the Karari archaeological occurrence¹⁴. An upper limit is set by dates of $1.32 \pm$

0.11 Myr and 1.22 ± 0.01 Myr for the Karari and Chari Tuffs, respectively. These dates are statistically indistinguishable and are believed to represent the same eruptive event. Technically, the lower age limit should be set by the age of the KBS Tuff, for which Fitch and Miller, using the $^{40}\text{Ar}/^{39}\text{Ar}$ step heating method, obtained a best estimate of 2.61 ± 0.26 Myr (ref. 14). Subsequently, Curtis *et al.* reported that they have made six conventional K-Ar determinations on samples from the KBS Tuff at the foot of the Karari Escarpment in Area 131. These values cluster closely around a mean of 1.8 Myr (ref. 15). The reasons for the discrepancy between the two sets of results are still under review. But, although the KBS Tuff is the marker horizon that forms the lowermost boundary of the stratigraphic interval containing the Karari Industry, most of the sites are contained in the strata at or above the level of the Okote Tuff complex and its equivalent at Ileret. Dates of 1.57 and 1.48 Myr for these tuffs, respectively, clearly provide the best indicators of the lower limits of the time interval in question.

Further chronological evidence has been obtained from palaeomagnetic stratigraphy¹⁶. The beds yielding the industry show predominantly reversed magnetic polarities and the few apparent normal results which were obtained at Ileret seem very likely to result from remagnetisation during the Brunhes Epoch. Since the Earth's field was reversed in the interval 1.2–1.6 Myr BP, the palaeomagnetic determinations for the Upper Member Beds are consistent with the K-Ar dates.

Biostratigraphic data are also concordant with these age determinations. On the basis of comparisons among various dated East African faunal samples, Maglio gave a best estimate of 1.3 Myr for the *Loxodonta africana* zone at East Rudolf, with the limits of uncertainty set at 1.0–1.6 Myr (ref. 13). More detailed work is being undertaken by J. M. Harris of the Kenya National Museum and his recent work does not suggest any need for major revisions in the biostratigraphic correlation of this part of the sequence, though the nomenclature will be changed (personal communication).

Characteristics of artefact samples

The overall characteristics of the series of artefact samples we have recovered can best be represented by the application of the typological system devised by Leakey¹⁷, though it has been necessary to add one or two categories. Table 2 presents a summary for each excavated assemblage of the percentage incidence of specimens in each artefact category.

Each assemblage comprises two fundamentally distinct classes of specimens: first, there are comparatively thick, massive boulders, cobbles and blocks of stone from which flakes have been removed by percussion, and second, there are relatively thin slivers of stone produced by conchoidal fracture. The first are termed 'cores', in the broadest sense of the term, and the second, 'flakes' and 'flake fragments'. Superimposed on this dichotomy is another important distinction: items which have been purposively shaped to particular patterns are set apart and referred to as 'tools', the word having a restricted technical sense. By contrast, the category 'debitage' contains 'flakes' and 'flake fragments' which have not been secondarily trimmed, plus those 'cores' that are regarded merely as by-products of flake production. This semantic distinction is not meant to imply that items ofdebitage were never used as implements. There are good reasons for believing that unretouched sharp flakes were functionally as important as the formally defined 'tool' category. Objects that are 'non-tools' in the above sense, but which show damage attributable to use are put in the third category often defined 'utilised', but we prefer the designation 'edge damaged' for this set.

Among early stone industries most of the cores (*sensu lato*) also seem to have been designed as 'tools' and at

coids and polyhedra occur with less frequency. A few bifaces and cleavers have been recovered from surface localities in circumstances that demonstrate conclusively their derivation from Upper Member beds. These tool forms, however, seem to be very rare in the material that is concentrated in occupation sites and have not yet been recovered by excavation.

The unbroken flakes show distinctive morphological features: small restricted platforms and diffuse bulbs of percussion. In particular, at sites associated with stream channels, battered cobbles and large boulder cores are present from which large well struck flakes had been struck. The trimming of the core tools must have also yielded numbers of flakes.

Comparisons and taxonomy

Very few datable artefact assemblages are known from the time span between 1 and 1.5 Myr BP. By far the largest and most detailed body of comparative data is that which derives from Bed II at Olduvai Gorge¹⁷. M. D. Leakey, who excavated the Olduvai material, recognised two main varieties among the samples that occur above the Lemuta Member: The Acheulian Industry and the Developed Oldowan Industry with the latter being subdivisible into phases A and B. Tables 2 and 3 show percentage frequency data for selected examples of Olduvai assemblages. Comparison of the two sets of data shows that not all the samples from the Upper Member of the Koobi Fora Formation are distinguishable from the Olduvai series; for example, FxJj 38 is not unlike FLKN level 5. But most Karari samples differed markedly, by virtue of the large percentages of core scrapers and other scrapers.

There are other contrasts. Polyhedra and discoids are more numerous at Karari sites but inhibit less battering and blunting of edges than is commonly evident in the Olduvai samples. The Karari assemblages lack spheroids and subspheroids, while these forms are present in relatively large proportions at most of the Bed II sites. The Karari Industry also contains core and cobble fragments with re-touch on the ventral face, which could not be accommodated in any of the typological categories proposed by M. D. Leakey. A separate class has been added to the type list to represent these.

Very few awls, burins or picks have been recovered from the Karari sites, whereas at Olduvai these forms occur more frequently, although in small quantities. A variable series of unstandardised trimmed pieces in both the Olduvai and Karari samples have been classified as 'sundry tools'.

Other assemblages from the age range in question should be mentioned briefly. The samples from the Humbu Formation at Peninj include large percentages of bifaces and are therefore unlike the Karari material^{18,19}. The tool component of the sample from Sterkfontein Extension site is dominated by choppers, polyhedra and spheroids and is therefore much more like the Developed Oldowan than the Karari Industry²⁰. Although the time relationships of the early material from North Africa are not known with certainty, sites such as Ain Hanech, or Sidi Abderhaman Ancien Exploitation level J may be of comparable age²¹. None of these assemblages includes significant numbers of core scrapers of Karari type.

In each comparison it is largely the morphology of the core scrapers that distinguishes the Karari series. The form range of other tool categories such as choppers, polyhedra and discoids, overlaps extensively with that of the equivalent sets in other assemblages. The rare bifaces that derive from the Upper Member are very like those of Olduvai Bed II, Peninj or the Lower Acheulian of North Africa.

It thus emerges that the morphological pattern shown by the assemblages from the Upper Member of the Koobi Fora Formation falls outside the known range of other described,

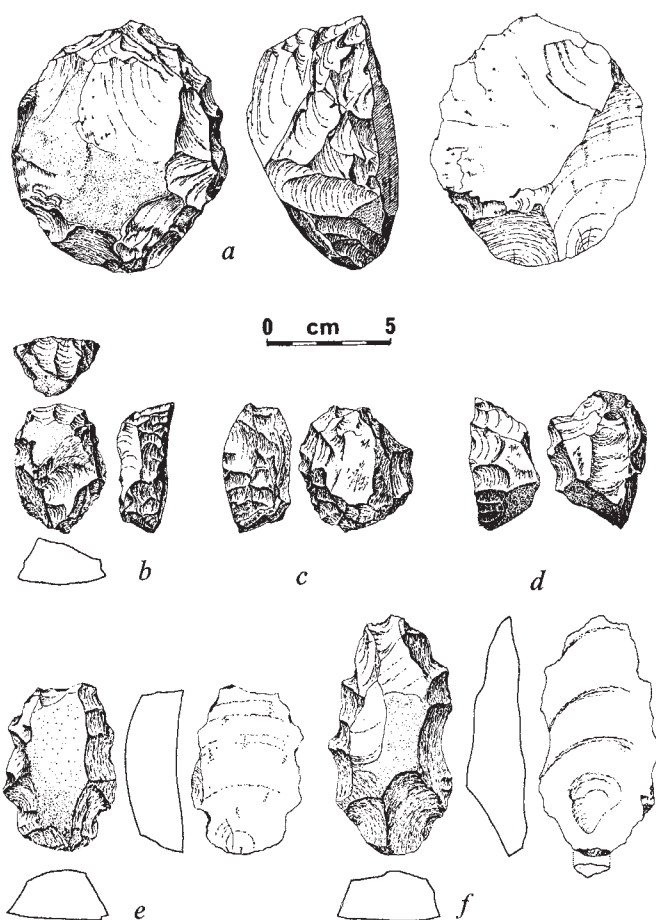


Fig. 3 *a*, Core scraper, FxJj 11; *b*, core scraper, FxJj 20 Main; *c* and *d*, core scrapers, FxJj 20 AB; *e*, core scraper, FxJj 20 IHS; *f*, light-duty scraper on flake, FxJj 18 NS. Made on lava. Drawings by Barbara Isaac.

contemporary classificatory sets. In accordance with the procedures proposed by the 1965 Burg Wartenstein Symposium²², and endorsed by the Sixth Pan-African Congress on Prehistory and the Study of the Quaternary, we propose to designate this distinctive series the Karari Industry.

As a formal definition we offer the following: the Karari Industry is a set of early Pleistocene artefact assemblages that shares broad, generalised features with other near-contemporary industries such as the Developed Oldowan Industry from Bed II at Olduvai, but which also shows distinctive patterns of differences from them. Generalised features held in common include the preponderance of opportunistic flaking technology in which highly organised, fixed patterns of core preparation are rare or lacking. Choppers, polyhedra and discoids are important components of the Karari Industry as in many other generalised industries. Handaxes and cleavers seem to have been a rare but significant part of the total artefact repertoire, as in the case of the Developed Oldowan B. The Karari Industry, however, is distinguishable from all other near-contemporary industries by the tendency of its component assemblages to include a conspicuous series of core scrapers. The percentage incidence of these is variable. A few assemblages, that clearly formed part of the material culture system that we are designating as the Karari Industry, show a low or negligible incidence of the key forms, but in most, where the sample is large enough, they are present in proportions well outside the range observed in other contemporary industries. In the Karari Industry even the scrapers that are not specifically core scrapers are distinct from those of most Oldowan assemblages in their tendency to be more massive.

Table 3 Composition of assemblages assigned to Karari Industry compared with selected assemblages from Olduvai Gorge Beds I and II

Sites	Overall composition*			Percentages of tool categories among tools												
	No. of artefacts	% Debitage	% Tools*	No. of tools	Core scrapers†	Flake scrapers†	Choppers	Protobifaces	Bifaces	Discoids	Polyhedrons	Sundry tools	Other tools	RCCF	spheroids	% All scrapers
Karari Scarp assemblages from flood plain silts																
FxJj 11	337	95.8	3.6	12			(33)			(8)	(17)		(25)	17		
17	247	98.8	0.4	1						(100)						
18 IHS	3267	96.2	3.5	114	21	17	2	3		25	21	6		5		38
20 M	2510	97.5	1.7	42	2	31	12	5		19	7		2	21		33
20 E	1211	96.3	3.0	36	8	25	31			22	8		3	3		33
20 AB	3462	98.7	0.7	25	20	20	16			4	8			32		40
Karari Scarp assemblages from channel sands and gravels																
FxJj 16	173	57.8	26.0	45	18	18	27	4		9	16			9		36
18 GSL	1556	87.1	10.9	169	20	17	19	2		15	19	3		5		37
18 GSU	214	86.9	10.7	23	22	30	17			4	17	9				52
18 NS	993	91.0	5.9	58	14	29	10	3		14	19		5	5		43
21 surface	54	85.2	11.1	6			(17)		(67)				(17)			
23	73	97.2	1.4	1			(100)									
38	115	58.2	34.8	40		2	(72)			5		2		(18)		2
Ileret assemblage from Lake Margin flood plain silts																
FwJj 1	318	99.4	0.3	1			(100)									
Olduvai, Oldowan																
FLK Zinj.	2470	92.1	2.4	60	15	30	28			5	15		7			45
FLKN 5	151	60.9	19.9	30	10		77			10					3	10
Olduvai, Developed Oldowan A																
FLKN SC	234	26.1	41.0	96		14	26			9	4	6	4		36	14
HWKE 3-5	1989	59.0	20.5	409	4	12	33	3		2	6	8	4		28	16
Olduvai, Developed Oldowan B																
BK II	6801	83.6	9.2	628	3	17	16		6	5	1		20		32	20
FC West	1955	76.7	10.5	206	11	9	30		4	4	3	4	2		34	20
TK	2150	90.1	4.9	106	6	24	6		14		1		21		29	29
Olduvai, Acheulian																
EFHR	522	77.6	17.4	91	3	3	15		54	9	6				10	7

The data for Olduvai Gorge are from ref. 18. Percentages for the tool types relative to the tool subset are rounded to the nearest whole number. Values for samples of less than 20 are shown in parentheses. RCCF, retouched core and cobble fragments.

* The other minor categories for which percentages are not shown are 'modified' and 'edge damaged' (= utilised).

† For the Olduvai analyses the distinction is between heavy duty and light duty scrapers.

If an associated set of excavated artefacts is needed as a type sample of the newly defined industry, we would nominate the archaeological material from the FxJj 18 Site Complex as suitable. (That completes the definition.)

The decision to name this material as a distinguishable industry is based solely on considerations of the morphology of the archaeological materials. We do not necessarily wish to infer the former existence of a 'cultural entity' or an ethnic entity that could be equated with the Industry. We also wish at present to avoid a definite judgement on the formal taxonomic relationships at any level of classification wider than that involved in grouping a set of related assemblages into an Industry. Clearly the material may be related in some way to the contemporaneous Developed Oldowan and Acheulian at Olduvai. It involves elements of both modes 1 and 2 in the schema of Clark²³.

Palaeoecological context and behavioural interpretations

An extensive archaeological survey has been carried out at Ileret and along exposures of the Karari Escarpment to gain some understanding of the early Pleistocene man-land relationships represented in the area. The distribution of sites in relation to the mosaic of former habitats is potentially informative of the ecological relationships of early

hominids. We found that they used the riverine floodplains and the alluvial fan deposits near the basin margins. Probably their range also included the valleys and hills of the more elevated surrounding terrain. These data are consistent with the idea that early hominids were mobile, opportunistic hunters and gatherers who used a wide range of resources.

More than 50 sites have been located and of these only two are in deposits laid down near the lake margins at Ileret, while the remainder are in varied alluvial deposits that formed 15-25 km inland from the shoreline. Of the 17 excavated sites nine are in direct association with the deposits of former stream channels while six are contained in the silty deposits of the broad floodplains that lay in between the stream channels. In addition to the excavated sites, we have located a further 40 archaeological occurrences through their surface traces, and these are potential sites for further investigation.

Several features emerge from an analysis of the pattern of distribution of the archaeological sites. Traces of past activities are found close to water sources, such as stream or river channels. This association has been observed elsewhere and may tentatively be explained by the fact that tree- and bush-lined stream channels would have provided shade and shelter as well as sources of nuts and berries. Ready access to raw material may have been another factor; in some cases, sites were located on gravel bars in stream

channels in close proximity to patches of basalt cobbles and boulders.

Fossil bone is rather rare and poorly preserved in the deposits containing the Karari Industry. But sealed 'living floors' that contain stone artefacts plus small quantities of bone fragments, generally in a highly comminuted state, do occur chiefly in floodplain contexts. Detailed study of bone samples is in progress.

Hominid remains occur interstratified with archaeological sites of the Karari Industry. A hominid mandible, KNM-ER 3230 (*Australopithecus cf. boisei*) was recovered by excavation 10–15 cm stratigraphically above the artefact-bearing horizon at FxJj 20²⁵. In area 130, KNM-ER 1805 (*Homo* sp. ?) and KNM-ER 1806 (*Australopithecus cf. boisei*) were recovered 40 m from FxJj 38 on the same stratigraphic horizon within channel deposits⁶. A wealth of hominid fossils, including specimens assigned to *Australopithecus* (cf. *boisei*) and *Homo* have been recovered in beds of the Ileret Member^{1–6}.

The discovery of the Karari sites has added significantly to knowledge of artefacts that are more than 1 Myr old. Well dated, excavated samples were previously available only from Olduvai¹⁷, Peninj^{18,19}, the Shungura Formation (Omo)²⁴, the Lower Member of the Koobi Fora Formation^{8,9} and perhaps Ain Hanach in Algeria²¹ and Sterkfontein in the Transvaal²⁰. Variety among this series is considerable and we suspect that as the sample is enlarged, an even greater range of differences will be discovered between sets of sites characteristic of the various regions in which evidence is preserved. Many factors could have interacted to cause variety; for example, differences in the characteristics of available stone; differences in particular, local needs for certain implements and perhaps fluctuating regional differentiation in habits. To distinguish the relative importance of these and other factors, we will need a far larger sample of assemblages that have been drawn from a varied spectrum of contexts, in each of several separate sedimentary basins. Our naming of the Karari Industry as an entity is not intended to prejudice the outcome of this kind of research; it simply reflects the fact that the material

from the Upper Member of the Koobi Fora Formation is distinctive and a clear unambiguous label is needed for it.

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Relationship between receptor and mammary tumour virus production after stimulation by glucocorticoid

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A short exposure of primary cultures of mouse mammary tumour cells to glucocorticoids results in at least a three-fold stimulation of mammary tumour virus (MTV) production. Specific interaction of glucocorticoids with the cytoplasmic and nuclear receptors can also be demonstrated. The biological potency of various steroids to stimulate MTV is related directly to the retention of the steroid-receptor complex in the nuclei. Progesterone has a high affinity for the cytoplasmic receptor, is not retained by the nuclei and does not stimulate or block the basal level of MTV production. It is, however, quite effective in abolishing the glucocorticoid-mediated stimulation of MTV and thus behaves as an antagonist of glucocorticoid.

CULTURES of cells and cell lines derived from primary mouse mammary tumours can be stimulated by glucocorticoids to produce increased quantities of mammary tumour virus (MTV) particles^{1–7}. The biological activity of glucocorticoid hormones is believed to be mediated through a specific interaction of the steroid with the steroid hormone receptors⁸. The presence of cytoplasmic and nuclear glucocorticoid hormone receptors in mouse mammary tumour cells have been demonstrated^{9–11}. A positive correlation has been reported between the binding of a steroid to the cytoplasmic glucocorticoid receptor and its biological potency in inducing MTV (ref. 11). These studies, however, failed to

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explain the role of progesterone in MTV synthesis because, in spite of its high affinity to the cytoplasmic receptor, it failed to stimulate MTV synthesis. Based on the observations that progesterone is an effective competitor for the cytoplasmic glucocorticoid receptor but cannot compete effectively with the glucocorticoid receptor-binding sites in the nuclei, we have postulated that progesterone is an antagonist of glucocorticoid action in mouse mammary tumour cells¹⁰. We now report the presence of cytoplasmic and nuclear glucocorticoid hormone receptors in cultured primary mouse mammary tumour cells; the acceleration of MTV production mediated by a short exposure to glucocorticoids as opposed to the continuous exposure to steroids used in previous studies, and an antagonistic action of progesterone on glucocorticoid-mediated stimulation of MTV.

Mouse mammary tumour cell cultures were prepared from spontaneous mammary tumours arising in BALB/cfC3HCr1 and C3H/Cr1 mice as described by McGrath¹. There was no significant difference between BALB/cfC3H and C3H cells with respect to either virus production or the effect of hormones on MTV synthesis. Dissociated cells were plated in 60-mm or 100-mm Petri dishes (Falcon) at a seeding density of 5×10^5 cells per cm^2 in Dulbecco's modified Eagle's medium containing 15% foetal calf serum (Gibco), penicillin (100 U ml^{-1}), streptomycin sulphate ($100 \mu\text{g ml}^{-1}$) and bovine insulin ($10 \mu\text{g ml}^{-1}$, Sigma). Cell cultures to be pulsed with steroids were changed to serum-free medium 24 h before the pulse, washed twice after the pulse and incubated for a further 24 h in serum-free medium. The resulting culture fluids were assayed for DNA polymerase activity.

For glucocorticoid receptor assays, all preparative procedures were carried out at $0-4^\circ\text{C}$. The cells were homogenised in known volumes of TESH buffer (0.01 M Tris , 0.0015 M EDTA , $0.012 \text{ M thioglycerol}$, $\text{pH } 7.4$). The homogenate was centrifuged at $800g$ for 10 min. The supernatant was designated the cytoplasmic extract. The nuclear pellet was resuspended and centrifuged once in phosphate-buffered saline and once in phosphate-buffered saline containing 0.25 M sucrose . The pellet was then extracted with TESH-KCl buffer (TESH buffer containing 0.4 M KCl , $\text{pH } 7.4$) and centrifuged and the supernatant extract was designated the nuclear extract. The binding reaction was performed at $0-4^\circ\text{C}$ by incubation of aliquots of cytoplasmic extracts with known concentrations of the radioactive steroid, either alone or in the presence of competing non-radioactive steroid. After incubation for 120 min. (unless otherwise stated), binding in the cytoplasmic extract was assayed using a Sephadex G-25 filtration technique previously described⁹ or by the DEAE-filter assay of Santi *et al.*¹². Binding capacity determined by the two procedures did not differ significantly. The total radioactivity present in washed nuclear pellets was extracted with 3 ml of ethanol and counted with 10 ml of scintillation fluid. Bound and unbound labelled steroid in nuclear extracts was separated by gel filtration on Sephadex G-25 columns using TESH-KCl buffer.

The DNA polymerase activity present in the tissue culture fluid from tumour cell cultures was processed as previously described^{2,13}. Assays were performed in reaction mixtures containing 50 mM Tris-HCl buffer, $\text{pH } 8.3$, 40 mM KCl , 10 mM MgCl_2 , $0.04 A_{260}$ units of poly(rC)-oligo(dG)₁₂₋₁₈ (P. L. Biochemicals) and $5 \mu\text{Ci}$ of ^3H -deoxyguanine triphosphate (9.3 Ci mmol^{-1}) at 37°C for 40 min. Assays were also performed using reaction mixtures containing Tris-HCl buffer, $\text{pH } 7.4$ and Mn^{2+} (0.5 mM).

To estimate DNA content, cell cultures were washed twice with Hanks balanced salts solution, scraped from the dish and precipitated with an equal volume of 10% perchloric acid (PCA) at 4°C . After 30 min the precipitates were collected by centrifugation at $1,600g$ for 10 min and washed twice with cold 5% PCA and twice with 70% ethyl

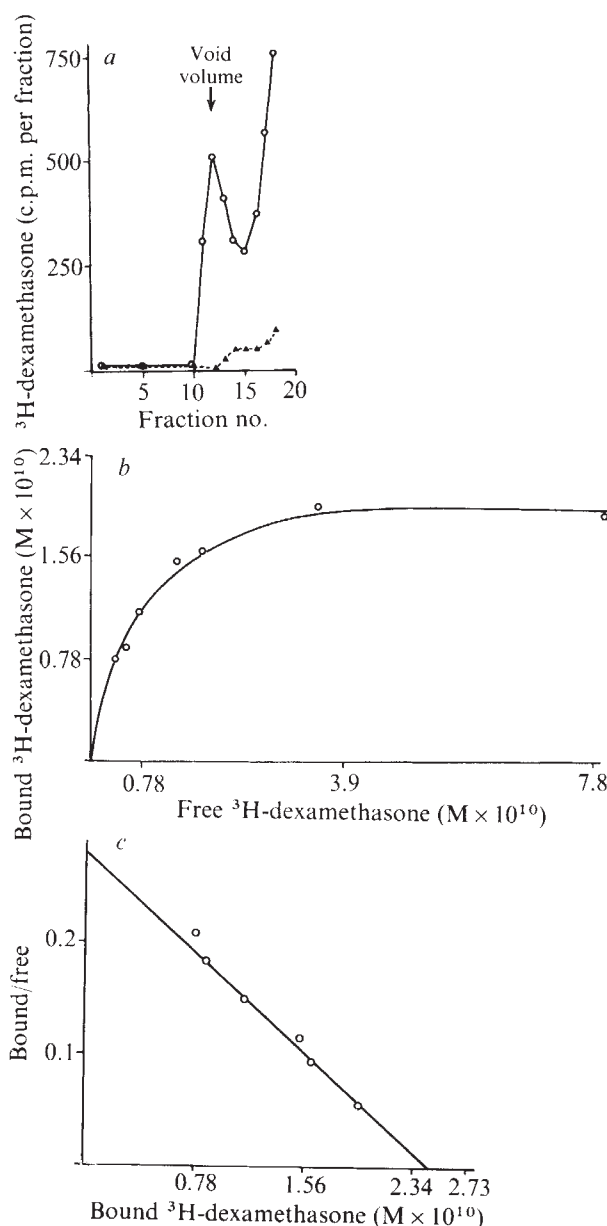


Fig. 1 (a), Binding of ^3H -dexamethasone in the cytoplasm of cultured mammary tumour cells. Samples (0.4 ml) of cytoplasm were incubated with $1.7 \times 10^{-9} \text{ M}$ of ^3H -dexamethasone ($\circ$) or with $1.7 \times 10^{-9} \text{ M}$ of ^3H -dexamethasone and 10^{-7} M unlabelled dexamethasone ($\blacktriangle$) at 4°C for 2 h. Bound radioactivity in each incubation was assayed on Sephadex G-25 columns. (b), Kinetics of specific cytoplasmic binding of ^3H -dexamethasone. Samples of cytoplasmic extracts were incubated with various concentrations of ^3H -dexamethasone with or without 100-fold excess of unlabelled dexamethasone for 2 h at $0-4^\circ\text{C}$. The data, represented as molar bound steroid in the total incubation, are plotted as a function of free-steroid concentration at equilibrium. The free steroid was determined by subtracting the bound steroid from the total steroid present in the incubation mixture. (c), Scatchard plot of the binding data from (b).

alcohol: 30% diethyl ether, and the pellet was allowed to dry. The pellet was dissolved in 1 N NaOH and left at 20°C overnight, neutralised with HCl and reprecipitated with an equal volume of 10% PCA at 4°C . The precipitate was washed twice with 5% PCA and hydrolysed in 5% PCA at $85-90^\circ\text{C}$ for 30 min. After hydrolysis the samples were mixed, centrifuged at $1,600g$ for 10 min and aliquots were assayed for deoxyribose content using the method described by Burton¹⁴. Hydrolysed calf thymus DNA was used as a standard.

Strong interactions of ^3H -dexamethasone with the macromolecules of the cytoplasmic extract of mammary tumour cells in culture were revealed by gel filtration analyses (Fig. 1a). Specific binding was eliminated when the extract was incubated with labelled dexamethasone in the presence of an excess of unlabelled dexamethasone. This indicates that there was only a limited number of binding sites for dexamethasone. The amount of specifically bound ^3H -dexamethasone in the cytoplasmic extracts of cultured cells as a function of the free steroid concentration is shown in Fig. 1b. Saturation of the binding sites was reached when the free steroid concentration was about 4×10^{-10} M. The specific binding data were also plotted by the Scatchard¹⁵ technique (Fig. 1c) and yielded a single straight line, indicating that only a single class of binding site was present. The equilibrium dissociation constant of the binding of the steroid to the receptor was estimated to be 8.8×10^{-10} M. The average dissociation constant as determined from four separate experiments using various concentrations of cytoplasmic extract was estimated to be 1.6×10^{-9} M. On the basis of the Scatchard plot, it can be assumed that 1 mol of steroid is bound to 1 mol of receptor. Thus, assuming 6.6×10^{-6} μg of DNA per cell nucleus, based on the average DNA content of the tumour cells in culture (0.015 μg DNA per 10^7 cells), there are about 1,800 sites per cell. The average number of binding sites per cell as determined from four

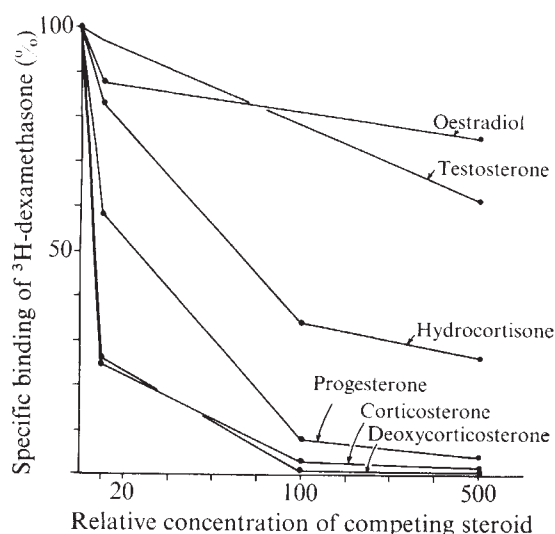


Fig. 2 Effect of various unlabelled steroids on binding of ^3H -dexamethasone to cytoplasmic extracts of mammary tumour cells in culture. Aliquots of cytoplasmic extracts were incubated with 10^{-9} M ^3H -dexamethasone alone or with various concentrations of non-radioactive steroids at 4°C for 2 h. Binding was measured by DEAE-filter assays as described in the text. Non-specific binding has been subtracted.

separate experiments was estimated to be approximately 1,400. Binding activity was destroyed by incubating cytoplasmic extracts with trypsin ($500 \mu\text{g ml}^{-1}$) or Pronase ($100 \mu\text{g ml}^{-1}$). Similar treatment, but with DNase (bovine pancreas $100 \mu\text{g ml}^{-1}$) and RNase ($100 \mu\text{g ml}^{-1}$), had no effect on the binding. Therefore the cytoplasmic receptors seem to be proteins. Various non-radioactive steroids were tested for their ability to compete with ^3H -dexamethasone for the receptor binding (Fig. 2). The order of affinity of various steroids to the cytoplasmic receptor was dexamethasone > deoxycorticosterone > corticosterone > progesterone > hydrocortisone.

It has been proposed that the effect of glucocorticoids in target tissues results from steroid-mediated interaction of the receptor with the genome¹⁶. To examine this phenomenon in mammary tumour cell cultures, we made parallel

studies on the nuclear localisation of dexamethasone and its stimulation of MTV. The data on the specific nuclear binding of steroids, and the subsequent stimulation of MTV in primary cultures of mammary tumour cells are shown in Fig. 3. The specific nuclear binding of dexamethasone was nearly complete by 30 min (Fig. 3a); as with other steroid receptor systems, nuclear binding was derived from the receptor in the cytoplasm because the total receptor binding in the cells remained constant throughout the experiment. The specific binding of ^3H -dexamethasone in the nuclei reached saturation with 10^{-8} M ^3H -dexamethasone in the incubation medium (Fig. 3b). As with fresh tumour slices^{9,10}, 10–20 times more than a saturating amount of steroid with respect to cytoplasmic receptor was required to achieve nuclear saturation. About 90% of the nuclear bound radioactivity could be solubilised and the radioactivity in the nuclear extract was bound to a macromolecule (Fig. 3c). Pulsing the cells with dexamethasone for 30 min was also sufficient to evoke a subsequent near maximal stimulation of MTV (Fig. 3d). The effect on MTV was most pronounced in the cultures assayed between 12 and 24 h after exposure to the steroid as opposed to earlier (0–12 h) or later (24–36 h) (data not shown). The dose-response curve for dexamethasone in stimulating MTV is presented in Fig. 3e, and Fig. 3f represents a plot of the relative potency of a given steroid to stimulate MTV and the amount of that steroid specifically bound in the nuclei.

Since mouse cells contain endogenous type C oncoviruses which after induction may or may not be stimulated by glucocorticoids^{7,17–19}, the cultures were also monitored for the presence of type C virus, using the divalent cation preference of the DNA polymerase reaction. The MTV-DNA polymerase will utilise the template-primer poly(rC)-oligo(dG)_{12–18} 70 times more efficiently with Mg^{2+} as the divalent cation than with Mn^{2+} , whereas murine type C viruses (Moloney MSV/MuLV) show a greater efficiency with manganese (our unpublished observations)^{7,20,21,22}. Furthermore the DNA polymerase activity in the tissue culture fluid from mammary tumour cells was shown to be associated with the MTV virion by biophysical, morphological and immunological criteria². In all the experiments described, parallel assays had been performed to determine the presence of type C viruses and no evidence was found for significant type C virus production. Furthermore, in some experiments, culture virus was also analysed for its viral polypeptide composition and in all cases only the MTV-specific polypeptide components were present (data not shown). Thus we are reasonably assured that the viral polymerase activities reported here represent the activity due to MTV and also reflect the virus production.

Previous studies from this laboratory¹⁰ have demonstrated that the binding of progesterone to the glucocorticoid receptor of mouse mammary tumours does not lead to the translocation of the receptor to the nucleus and this was also true with tumour cells in culture (data not shown). As shown in Fig. 3, the nuclear-bound steroid is closely related to the stimulation of MTV, and therefore as suggested previously¹⁰, it is possible that progesterone acts as an antagonist to the glucocorticoid-mediated stimulation of MTV. The results of the following experiments demonstrate that this may indeed be true. Exposure of cell cultures to progesterone alone for 30 min did not stimulate MTV but neither did it block the basal level of MTV production (Table 1). This agrees with earlier observations made with progesterone on long term cultures of murine mammary tumour cells^{2,7}. If cells were exposed to dexamethasone in the presence of an excess of progesterone, however, there was a reduction in the stimulation of MTV due to dexamethasone (Table 1). The average of three experiments on primary culture of tumour cells indicates that when a 100-fold excess of progesterone is added with dexamethasone, there is a 70% inhibition of the dexamethasone-mediated

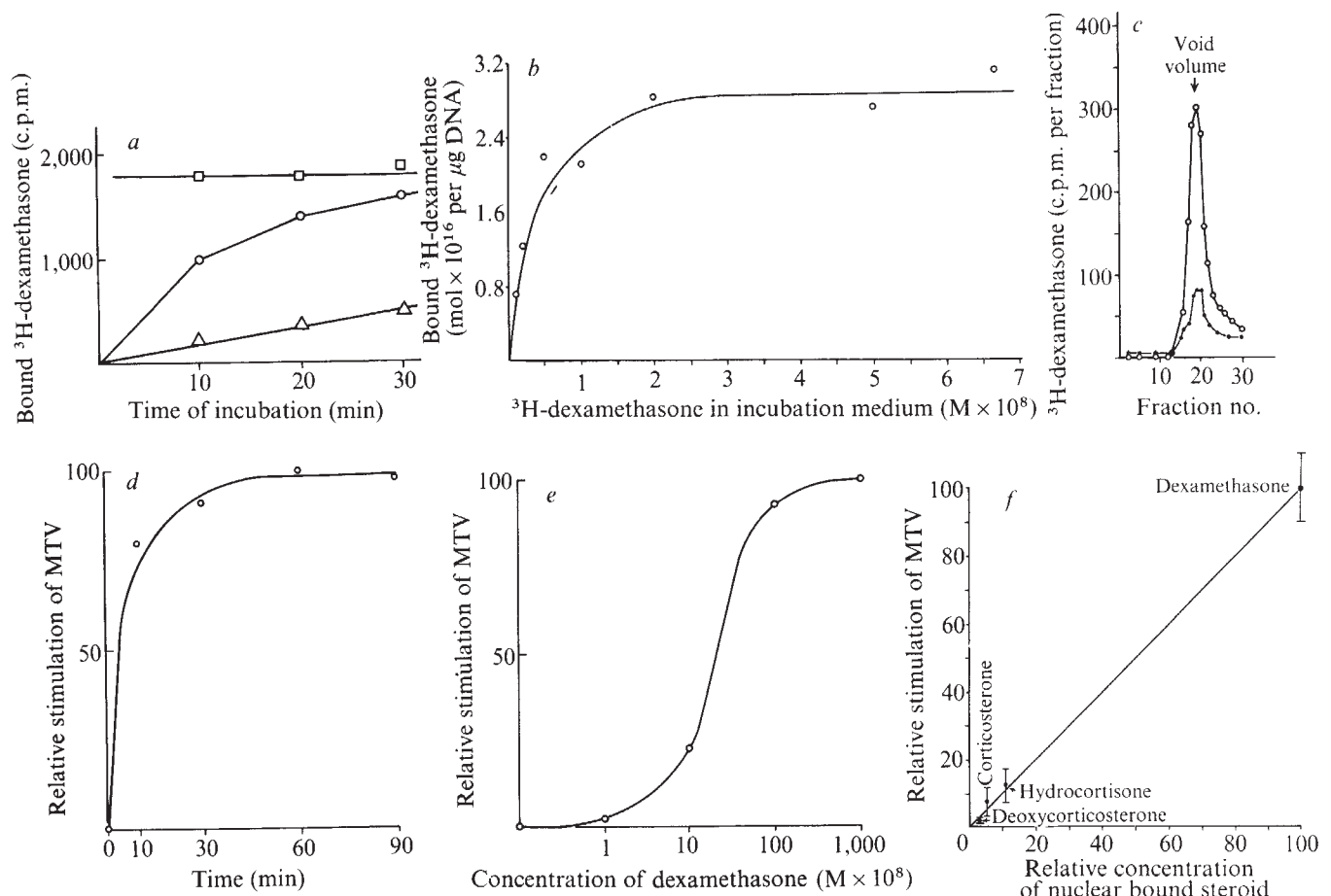


Fig. 3 Nuclear binding of ^3H -dexamethasone and its stimulation of MTV. In (a)–(e), to estimate binding to receptor, the cells were incubated at 37°C with specific concentrations of ^3H -dexamethasone for specified times. Parallel sets of incubations were done with 100-fold excess of non-radioactive dexamethasone to determine nonspecific binding. At the end of the incubations, cell fractions were prepared and assayed for binding. The rate of MTV synthesis was estimated by assaying for DNA polymerase activity in the tissue culture fluid from duplicate cultures of tumour cells 24 h after exposure to steroid. (a), Cells were incubated with 10^{-8} M ^3H -dexamethasone with or without 100-fold excess of unlabelled dexamethasone. $\circ$, Nuclear binding; $\square$, specific nuclear and cytoplasmic binding; $\triangle$, nonspecific nuclear binding. (b), Cells were incubated with various concentrations of ^3H -dexamethasone for 30 min. (c), Gel filtration on Sephadex G-25 columns of nuclear extracts of tumour cells in culture labelled with ^3H -dexamethasone only ($\circ$) or also with 100-fold excess of unlabelled dexamethasone ($\bullet$). (d), Tumour cells were treated with dexamethasone for the times indicated, and culture fluid was assayed 24 h after exposure to steroid. (e), Cells were exposed to various concentrations of dexamethasone for 30 min and culture fluids were assayed after 24 h for DNA polymerase activity as indicated in the text. (f), Plot of the relative concentration of nuclear receptor-bound steroid and its relative stimulation of MTV. To estimate stimulation of MTV, cells were pulsed with 10^{-6} M of various steroids for 30 min and the tissue culture fluid was assayed 24 h later for viral DNA polymerase activity. To estimate nuclear receptor binding, the cells were treated with 10^{-8} M of various labelled steroids for 30 min, at the end of which binding assays were performed. Parallel cultures were treated also with 10^{-6} M unlabelled dexamethasone. Nonspecific binding was subtracted. The data are therefore due to the specific binding of the labelled steroid to sites specific for glucocorticoid receptor only. In each case the data were calculated as pmol nuclear-bound steroid per mg DNA. The data thus obtained for MTV and nuclear binding with each steroid is expressed as relative to that obtained with dexamethasone. The average stimulation of MTV due to dexamethasone was 2.5 times the basal level. The results are the average of six separate determinations $\pm$ s.d.

stimulation of MTV. This supports the notion that progesterone can antagonise the glucocorticoid-mediated stimulation of MTV. Table 1 also shows that to observe a significant antagonistic effect of progesterone on MTV stimulation, the levels of progesterone must be at least 100 times in excess of that of added dexamethasone. This is necessary since the affinity of progesterone to the glucocorticoid receptor is less than that of dexamethasone and hence binding of progesterone to receptor in levels equivalent to dexamethasone occurs only at higher concentrations of progesterone. Treatment of cells for short times with concentrations of progesterone as reported here do not result in any toxicity. This was ascertained by measuring the overall incorporation of labelled amino acids into total cell protein by the tumour cells at the time when the culture fluids were assayed for DNA polymerase activity (data not shown).

The ability of various steroids to stimulate MTV in cultured murine mammary tumour cells has been reported¹⁻⁷

and our studies confirm this for primary cultures of mammary tumour cells, and demonstrate that a 30-min exposure to steroids is sufficient to elicit the response at 24 h. Our studies show that cytoplasmic and nuclear glucocorticoid hormone receptors are present in tumour cells in primary culture and, as with other steroid hormone receptors, binding of the steroid to the cytoplasmic receptor is an initial event responsible for the subsequent steroid binding to the nucleus. Also, all steroids that bind to the cytoplasmic receptor of the mammary tumours are not retained by the nucleus, and progesterone falls into this category. This interaction of progesterone with the glucocorticoid receptor binding to nuclei has been described in detail¹⁰. In the studies reported here we have also demonstrated a direct relationship between the kinetics of the nuclear binding of a steroid and its subsequent stimulation of MTV. In addition, we have shown a quantitative correlation between the retention of the steroid-receptor complex in the nuclei as

measured 30 min after exposure to steroid and its ability to stimulate MTV. Therefore, even though progesterone has a high affinity for the cytoplasmic receptor, its failure to remain bound to the receptor in the nucleus can lead to its inability to stimulate MTV. Similarly when used at appropriate concentrations with dexamethasone, it can block the stimulation of MTV by competing with dexamethasone for the cytoplasmic receptor sites and preventing its retention in the nucleus. Thus the data reported here are consistent with the role proposed for progesterone as an antagonist of glucocorticoid action in mammary tumours¹⁰ and in hepatoma cells²³.

Table 1 Progesterone antagonism of dexamethasone-stimulated MTV production

Steroid treatment	Relative stimulation of MTV production $\pm$ s.d.
Dexamethasone (10^{-6} M)	100 $\pm$ 8.9% $n = 6$
Dexamethasone (10^{-6} M) + progesterone (10^{-4} M)	31.2 $\pm$ 5.6% $n = 6$
Dexamethasone (10^{-6} M) + progesterone (10^{-5} M)	89.8 $\pm$ 15% $n = 4$
Progesterone (10^{-4} M)	3.25 $\pm$ 5.2% $n = 4$
Progesterone (10^{-5} M)	2.75 $\pm$ 2.5% $n = 4$

The results are expressed as % of the dexamethasone-stimulated DNA polymerase activity above the basal level. Cell cultures were pulsed for 30 min with the steroid combinations and the culture fluids were collected 24 h later and assayed for enzyme activity. The average incorporation of the Mg^{2+} -stimulated DNA polymerase activity at 100% was 25,400 c.p.m.

Based on the observed relationship between the retention of the steroid-receptor complex in the nuclei and the subsequent stimulation of MTV, some aspects of the steroid-mediated MTV synthesis can be explained. (1) Although a single, 30-min pulse with steroid is sufficient to translocate the receptor from the cytoplasm to the nucleus and to stimulate the subsequent MTV synthesis nearly threefold, when measured 24 h later, continuous exposure of the cells to the steroid is necessary to maintain the full level of MTV synthesis. This may be because it is necessary to retain in the nuclei optimal levels of steroid-receptor complexes for periods longer than 30 min. But when the steroid is removed from the medium at the end of the pulse, the equilibrium of the subcellular distribution of the steroid-receptor complex can be affected, causing some dissociation of the steroid from the complex in the nuclei. Since a quantitative relationship seems to exist between nuclear binding and MTV synthesis, this can lead to a decreased level of MTV synthesis. In this context it is important to mention that during the 24 h after the steroid pulse and washes, about 10^{-10} M steroid is released from the cells into the incubation medium. In separate experiments where the cells were exposed continuously to 10^{-10} M steroid, at the end of 24 h there was no detectable increase in the viral polymerase activity (data not shown). Thus the observed stimulation of MTV polymerase activity must result from the steroid-receptor complex formed during the pulse and not from the continuous presence of the low level of residual steroid in the medium. (2) Our dose-response curve for dexamethasone-mediated stimulation of MTV indicates that to obtain maximal stimulation, 10^{-6} M dexamethasone is needed while 10^{-8} M dexamethasone seems to be sufficient to saturate most of the receptor sites. It is important to remember, however, that the cells were pulsed with the steroid only for 30 min while the response was measured 24 h later. Thus, it is possible that with 10^{-6} M dexamethasone, there is a near maximal saturation of binding sites and a relatively smaller decrease in the dissociation of the steroid-receptor complex from the nuclei on withdrawal of the steroid at 30 min. This increase

in the amount of steroid retained by the nuclei after withdrawal of 10^{-6} M dexamethasone as opposed to 10^{-8} M dexamethasone may be just enough to maintain maximal synthesis of MTV. This may be the reason why, with 10^{-5} M dexamethasone, there is no further increase in MTV synthesis. It is also likely, however, that the dose-response relationships of steroid binding and steroid-mediated MTV synthesis do not fit, and elucidation of this problem must await further studies. (3) Our data show that while deoxycorticosterone has at least as high an affinity as hydrocortisone for the cytoplasmic receptor, it is able to stimulate MTV only suboptimally. As reported earlier¹⁰, even with saturation of all the receptor sites in the cytoplasm with deoxycorticosterone, there is only partial translocation, or retention, of the receptor to the nuclei and this may explain why this steroid is only suboptimally active, for even at high concentrations it cannot lead to maximal stimulation of MTV².

The mechanism by which steroids are believed to influence gene expression in target cells involves the binding of the steroid to the cytoplasmic receptor and its subsequent interaction with DNA and chromatin, the net result of which leads to the expression of the phenotypic effect. The data on the glucocorticoid receptor and MTV synthesis obtained with various steroids seem to fit this model. As reported here and elsewhere¹⁰, specific binding of glucocorticoids to cytoplasmic and nuclear components occurs in mouse mammary tumours. As shown here, the interaction of the receptor with the nuclear components seems to be crucially related to MTV synthesis. The accumulation of MTV-specific RNA seems to be a primary response to the steroid²⁴ and reaches maximum at 6 h and finally as shown here, an effect on MTV synthesis can be demonstrated at 24 h. In this respect, the glucocorticoid-mediated stimulation of MTV is very similar to some of the most carefully studied examples of enzyme induction by steroids²⁵⁻²⁷.

Finally, a comment is needed about the nature of glucocorticoid-mediated synthesis of MTV. All data reported so far indicate that the steroid-mediated phenomenon is an amplification of the MTV synthesising apparatus rather than a true induction. This argument is based on the fact that the cells in culture synthesise MTV even in the absence of added steroid and steroids are required only for maximal production. Furthermore, progesterone does not inhibit the basal level of MTV but only that which is mediated by the glucocorticoid. Our studies, however, cannot provide any information with respect to particular sequences involved in the cell-virus interaction which are amplified by the hormonal control. Further investigations are also necessary to see if the primary site of hormone action lies in the host cell or the integrated virus in the host cell.

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4S *oop* RNA is a leader sequence for the immunity-establishment transcription in coliphage λ

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oop RNA, which is initiated at the p_o promoter and is 81 nucleotides long, can function as a leader sequence for the λ immunity establishment transcription, previously believed to originate at a special promoter p_{re} located in the y region. Thus, *oop* RNA seems to have a dual role, either favouring the lytic cycle as a primer for the initiation of λ DNA replication, or leading to the establishment of lysogeny when elongated into the *imm* transcript, which directs synthesis of the repressor.

To establish and maintain the prophage state, phage λ directs synthesis of the repressor protein, the product of gene *cI* (ref. 1). There are two known modes of repressor synthesis: (1) In the establishment mode, observed early after λ infection, the *cI* transcription is believed to originate at the p_{re} promoter, hypothetically defined by the *cY* mutations located in segment y just to the right of gene *cro* (Fig. 1), and is positively regulated by genes *cII* and *cIII* (refs 2-5). (2) In the maintenance mode, characteristic of the established prophage, the *cI* transcription originates at the p_{rm} promoter (originally designated p_c promoter⁶) just to the left of gene *cro* (Fig. 1) and is both positively and negatively controlled by the *cI* product^{2,3,7}. Both the maintenance and establishment modes of the *cI* transcription are under the negative control of gene *cro* (refs 1-6).

A small RNA species designated *oop* RNA (ref. 8) is synthesised a short distance upstream from the hypothetical location of the p_{re} promoter. Synthesis of *oop* RNA, which is believed to serve as primer for the initiation of λ DNA synthesis, depends on several phage and host replication functions⁹. Since establishment of lysogeny also depends on some replication functions^{2,3,10} and because of the close proximity between the hypothetical location of p_{re} and the site of *oop* RNA synthesis, we sought to establish some relationship between these two entities. Moreover, the isolation and mapping of the *sar* mutations, which affect λ DNA replication but stimulate repressor synthesis overcoming the *cY* defects, suggested that the establishment mode of repressor transcription might originate at the promoter, designated *pro*, between genes *cII* and *O* (ref. 10).

Specifically, we postulated that *oop* RNA, in addition to serving as the primer for DNA replication, can also have the role of a leader sequence that can be extended into the *imm* region by some anti-termination function, most likely depending on the *cII* and *cIII* products. This would imply that the p_{re} promoter, as defined by the *cY* mutations², either does not exist or has only a minor role, whereas the *Pre* function—that is, promotion of the establishment mode of *cI* transcription, is really carried out by the p_o promoter of the *oop* RNA (ref. 8). As will be discussed later, the *c42* and similar *cis*-dominant mutations in the y segment, which were thought to define the

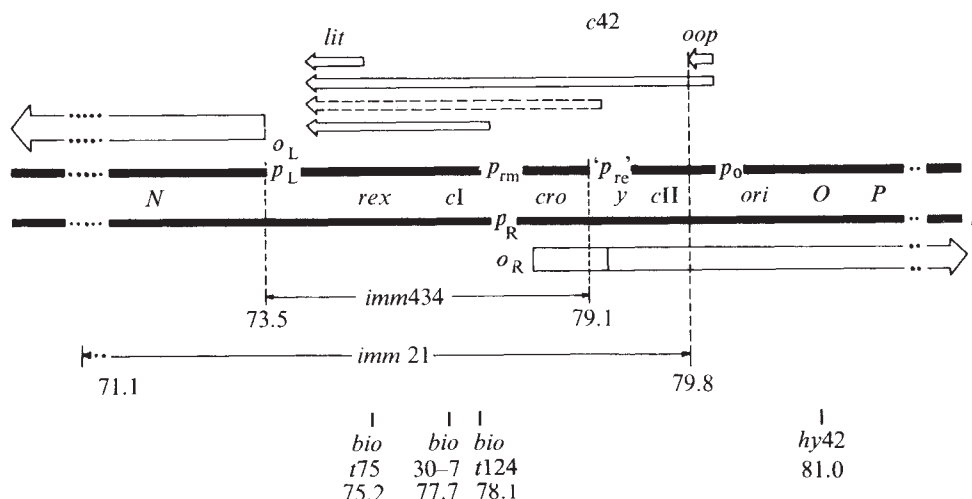
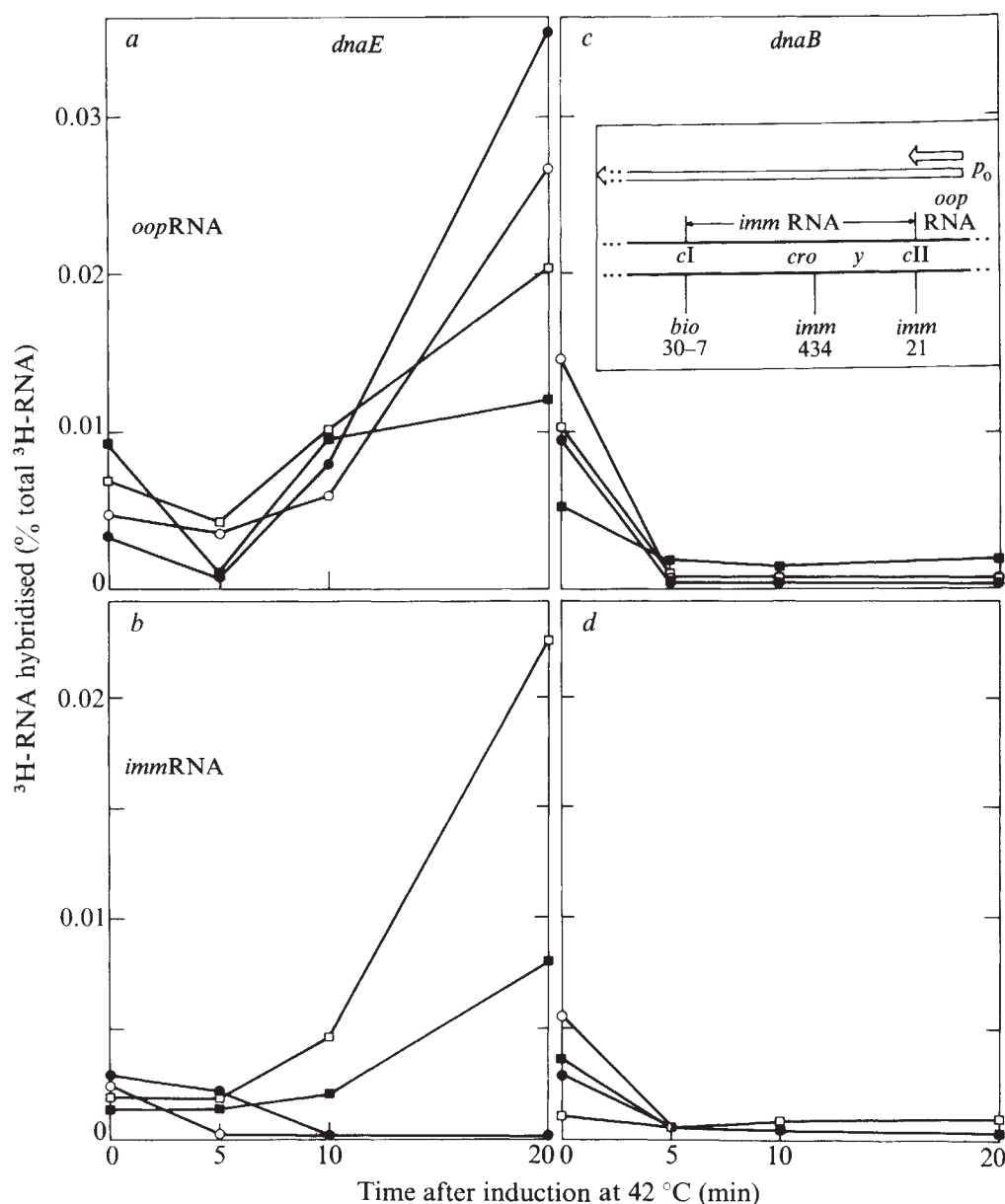


Fig. 1 Genetic and physical map of the immunity proximal region of phage λ . The *l* and *r* strands of λ DNA are represented by heavy lines and the various transcripts by open arrows. The dashed line arrow corresponds to the repression establishment mode of transcription²⁻⁵, which was originally thought to be initiated in the y region². The p_o -*cI*-*rex* arrow (solid line) represents our current model for the establishment mode of the *cI*-*rex* transcription. For the designations of genes and other symbols, and for the positions of deletion endpoints (expressed in % λ units measured from the left terminus of mature λ DNA) see refs 6, 8 and 13-17. The promoters and operators are represented by the lower case letters *p* and *o*, respectively, with corresponding subscripts. The map is not drawn precisely to scale.

Fig. 2 Leftward transcription of the immunity and *oop* regions after prophage induction. The RNA was labelled for 1 min with ^3H -uridine either at 30 °C (zero time) or at the indicated times after induction at 42 °C. The extracted ^3H -RNA was first prehybridised to 1 strand of $\lambda\text{bio}30\text{-}7$ DNA (see Fig. 1 and inset in c), eluted, and then analytically hybridised with 1 strand of $\phi 80\lambda\text{imm}\lambda$ ($\text{hy}5$)¹⁸ and its derivatives⁸, $\phi 80\lambda\text{imm}434$ and $\phi 80\lambda\text{imm}21$ (for methods see refs 8, 11). The *oop* transcription (panels a and c) is calculated as percentage total ^3H -RNA input that hybridises to $\phi 80\lambda\text{imm}21$. The *imm* transcription (b and d) is similarly calculated as that ^3H -RNA which hybridises to $\phi 80\lambda\text{imm}\lambda$ minus that to $\phi 80\lambda\text{imm}21$. Data for the *dnaE*ts (JG55 = E486) and *dnaB*ts (JG28 = Hfr H165/70) lysogens^{9,19} are shown in a,b and c,d, respectively. The prophages were $\lambda\text{cI}857$ (○), $\lambda\text{cI}857\text{cro}27$, (□), $\lambda\text{cI}857\text{cII}68$ (●) and $\lambda\text{cI}857\text{cro}27\text{cII}168$ (■), (see refs 2-4).



p_{re} promoter¹⁻³, might actually correspond to either transcriptional blocks or one of the sites of the anti-terminating effects of the products of genes *cII* and *cIII*.

To prove that the p_o promoter can carry out the Pre function, two experimental approaches were used: one to explore the correlation between the *oop* and *cI* transcriptions, and the other to isolate and characterise the elongated *oop*-*cI* transcript.

Correlation between the establishment mode of *cI* transcription and synthesis of *oop* RNA

The ^3H -labelled, leftward-transcribed RNA was assayed by two-step analytical hybridisation¹¹ within the interval *cI*-*cro*-*y*-*cII* (establishment mode with exclusion of the complications due to the *lit* RNA; ref. 8) and in the *oop* region (see Figs 1 and 2). The experimental design included blockage of λ DNA replication so as to keep the gene dosage constant, inactivation of λ gene *cro* so as to eliminate the shifting expression of its various repressing functions, and thermal inactivation of the *cI*ts product to induce prophage, and to minimise the maintenance mode of *cI* transcription and both the positive and negative effects of the repressor^{2,3,7}. Because synthesis of *oop* RNA is known to depend on the λ replication functions *ori*, *O* and *P* and the host replication genes *dnaB* and *dnaG*, but

not on *dnaE* (ref. 9), the *dnaE*⁻ host mutation was used to block λ DNA synthesis (Fig. 2a and b). As controls, *dnaB*⁻ hosts (Fig. 2c and d) and P^- λ mutants were included. In other words, the kinetics of the leftward *imm* and *oop* transcriptions were studied after induction of $\lambda\text{cI}857$ prophage at 42 °C, in the absence of DNA replication and in *cro*⁻ compared with *cro*⁺ conditions.

In the *dnaB*⁻ condition (Fig. 2c and d) or for P^- prophage in *dna*⁺ host (to be published elsewhere) there is a perfect correlation between the *imm* transcription and *oop* RNA synthesis, since both are blocked after induction. This is not a general effect on transcription because the major leftward p_L transcription remains high (refs 11-12 and present data).

In *dnaE*⁻ lysogens the perfect correlation between the *imm* and *oop* transcriptions was only observed in *cro*⁻*cII*⁺ conditions (Fig. 2a and b). In *cro*⁺*cII*⁺ and *cro*⁺*cII*⁻ lysogens the *imm* transcription, but not *oop* RNA synthesis, is eliminated. A somewhat intermediate situation is observed for the *cro*⁻*cII*⁻ prophage.

These data enable the following conclusions to be made. Synthesis of the *cI* RNA after induction depends in the same manner as that of the *oop* RNA on host and phage replication functions, such as *dnaB* and *P*, respectively. The *cro* product,

an immunity-specific negative regulator, blocks selectively the *cI* transcription, thus decoupling it from the *oop* RNA synthesis. Not only does the *cI* transcription depend on the same factors as the *oop* RNA synthesis, but its full expression also requires the *cII* function. In *cro*⁻ conditions, some *cI* transcription is observed even in the absence of the *cII* function.

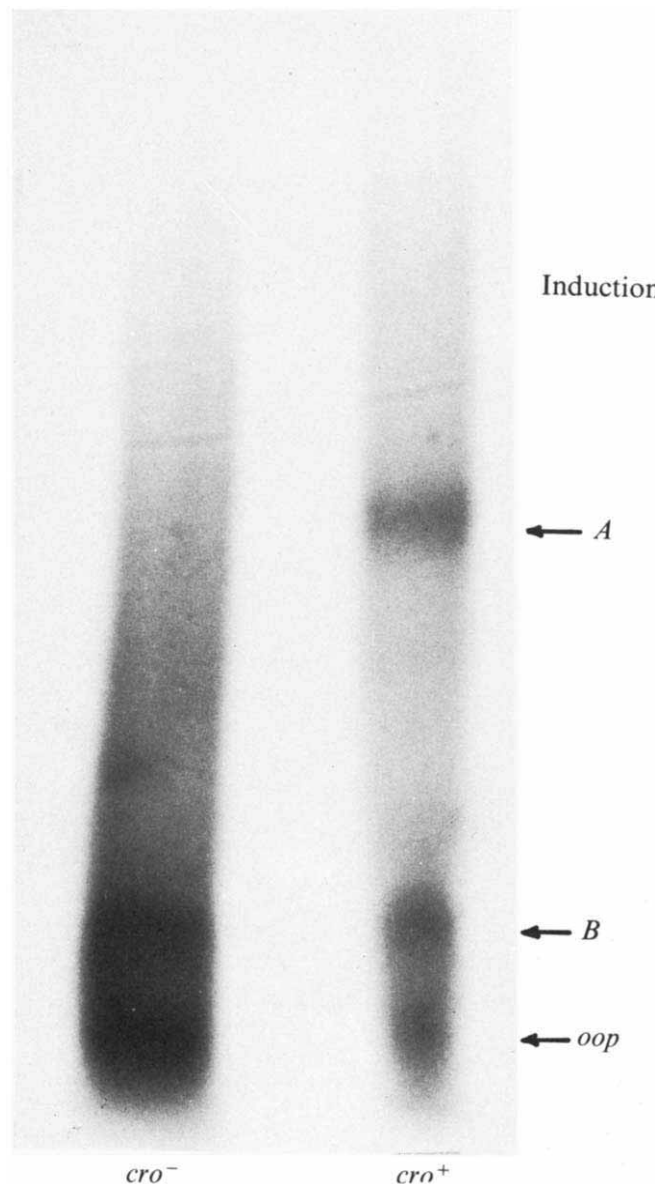


Fig. 3 Autoradiograms of λ RNA fractionated by polyacrylamide gel electrophoresis. The *dnaEts*(λ cI857*cro*⁺) and *dnaEts*(λ cI857*cro*⁻) lysogens were grown to $A_{575} = 0.35$ in MCGB medium¹¹ enriched with 0.4% yeast extract and 20 μ g thymidine ml⁻¹. The cells were spun down, washed twice, resuspended in 10 mM MgSO₄ at 5×10^8 cells ml⁻¹, shaken 60 min at 30 °C and transferred to 20 ml labelling medium containing 25 mCi ³²P and prewarmed to 42 °C (see ref. 21). The RNA labelled from 0 to 20 min at 42 °C was extracted, heated to 80 °C for 4 min to dissociate any double-stranded RNA, hybridised to *I* strands of λ bio30-7 DNA (4 h at 40 °C in 50% formamide), collected on B6 nitrocellulose filters (Schleicher & Schuell), and eluted as described in ref. 21. The eluted ³²P-RNA was ethanol precipitated (3 volumes, overnight at -20 °C) and then dissolved in 30 μ l buffer E (90 mM Tris, 8.9 mM H₃BO₃, 2 mM Na-EDTA, pH 8.3) containing 6 M urea, 0.1% bromophenol blue, and 20% (v/v) glycerol. The samples were layered on to a 3.5% polyacrylamide slab gel²², and electrophoresed for 8–10 h at 25 mA in buffer E. X-ray film (Kodak NS54T) was exposed for 45 min to slabs contained in polyvinyl chloride wrap.

These results show that the establishment mode of *imm* RNA synthesis after prophage induction could be an extension of the *oop* transcription. At least two-thirds of this *imm* transcription seems to depend on the *cII* product and is completely blocked by the *cro* product. To distinguish between two alternatives—that of a single extended *oop-cI* transcript against the possibility that the *oop* and *p_{re}-cI* RNAs represent two separate but jointly regulated transcripts—it was necessary to isolate and characterise the RNA synthesised in the *oop-cI* region.

Isolation and characterisation of the elongated *oop-cI* RNA

The leftward RNA transcribed in the *cI-oop* region was isolated in two steps. RNA labelled with ³²P for 0–20 min after thermal induction of *dnaEts*(λ cI857*cro*⁺) was first prehybridised in 50% formamide 40 °C to *I* strands of λ bio30-7 (Fig. 1), eluted, and then fractionated on a 3.5% polyacrylamide gel. The three major bands seen in Fig. 3a are: band *A* (about 9S), a plausible candidate for the *imm* RNA, band *B* (about 5S) probably originating in the *b2* region and not further characterised, and band *C* (about 4S) identified as *oop* RNA. Only RNA bands *B* and *C* are produced by the induced *dnaEts*(λ cI857*cro*⁺) lysogen (Fig. 3b). The startpoint of RNA corresponding to the band *A* was determined by transcriptional mapping⁸, and found to be located at about 80.1% λ units on the mature λ map, just to the right of the *imm21* boundary (Fig. 4). Since this site is also the startpoint of *oop* RNA (ref. 8), we conclude that band *A* is the elongated *oop* transcript corresponding to the establishment immunity transcript.

This conclusion was corroborated by several additional experiments.

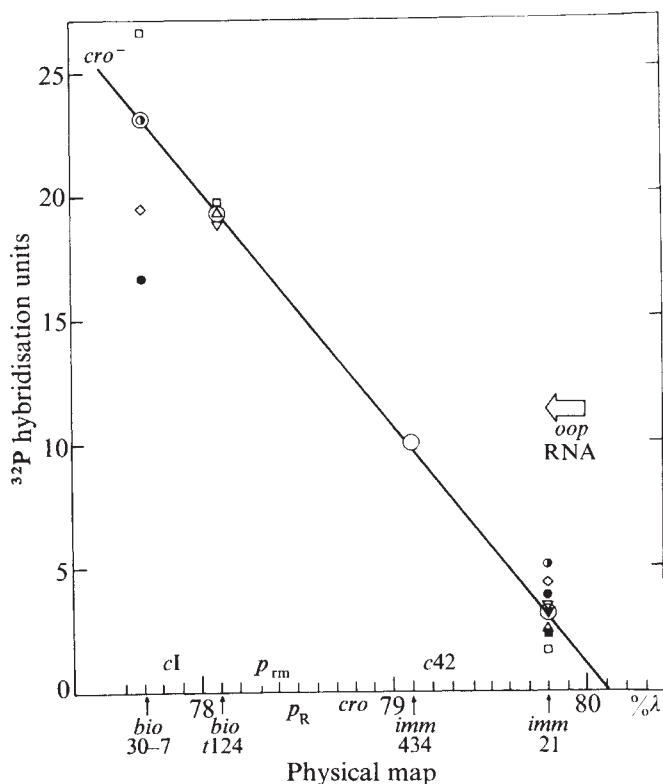
By prehybridisation to λ bio30-7 *I* strands followed by RNase treatment, the *oop-cI-rex* establishment immunity RNA was deliberately truncated to about 1,070 bases (2.3% λ units from the startpoint of *oop* to the *bio30-7* endpoint; (see Fig. 1) which enabled it to enter the 3.5% polyacrylamide gel. The behaviour of this *oop-imm* transcript could be changed by either shortening it to about 900 bases or lengthening it to about 2,150 bases by prehybridisation to λ bio124 or λ bio75 *I* strands, respectively (Fig. 1). As expected, prehybridisation to λ bio124 increases slightly the mobility of band *A* (gel not shown). The shortened transcript has the same startpoint at about 80% λ (Fig. 4). On prehybridisation to λ bio75, the resulting RNA does not enter the 3.5% gels, but forms a band in the 2% polyacrylamide gel reinforced with 0.5% Agarose (gel not shown).

The complete absence of band *A* in the 3.5% gel and the presence of only one band *A* in the 2% gel after prehybridisation to the *I* strand of λ bio75 DNA proves that band *A* (Fig. 3) corresponds to a single *oop-cI* RNA species, and not to two separate *oop-cII* and *p_{re}-cI* transcripts, fortuitously of roughly equal length.

As an alternative to the gel fractionation step, the *imm* RNA was also isolated as material excluded from the Sephadex G-75 column. As shown in Fig. 4, the startpoint of this RNA is also at about 80% λ .

Although we did not observe any *imm* RNA after induction of λ *cro*⁺ lysogens, it is known that the immunity establishment pathway is observed early after infection with λ *cro*⁺ phages^{2, 3}. We therefore looked for band *A* after infection of *Escherichia coli* *dnaE*⁻ with λ cI857*cro*⁺. Band *A* (gel not shown), which corresponds to the joint *oop-cI* transcript as determined by transcriptional mapping (Fig. 4), is produced early after infection with either λ *cro*⁺ or λ *cro*⁻ but not with λ *cro*⁺*cII*⁻ or λ *cro*⁻*cII*⁻. The appearance of the *oop-imm* RNA early after infection but not after induction (Figs 3 and 4) might reflect the presence of some prophage products, possibly *rex* or traces of *cro*, at the time of induction, whereas after infection they might reach the critical concentration only after some delay.

Fig. 4 The transcriptional mapping of the startpoint for the physically isolated immunity establishment RNA. ^{32}P -RNA corresponding to band A (see Fig. 3a) was isolated from the corresponding slice of the polyacrylamide gel by extraction with NTE buffer (0.3 M NaCl, 10 mM Tris-HCl, pH 8.1, and 1 mM Na.EDTA) for about 10 h at 4 °C, followed by ethanol precipitation of the RNA and redissolving in 1 ml NTE buffer. This RNA was hybridised to 10 μg / strand DNA of $\phi 80\lambda\text{imm}\lambda$ ($\phi 80\text{att}80\text{imm}\lambda$; hy5) and its *imm434* and *imm21* derivatives specified in Fig. 2, as well as of $\phi 80$, $\phi 80\text{hy}42$, $\lambda\text{rep}22$ and $\text{P}22\text{rep}\lambda$, in 0.4 ml NTE buffer containing 50% (v/v) formamide, at 40 °C for 4 h. After addition of 10 μg tRNA carrier, the nucleic acids were precipitated with 3 volumes ethanol (−20 °C, overnight), spun down, and redissolved in 0.4 ml NTE buffer. After addition of 0.4 μg pancreatic RNase, the solution was incubated for 15 min at 37 °C, supplemented with 10 ml $2\times\text{SSC}$, slowly filtered through B6 filters (see Fig. 3), and the radioactivity of the ^{32}P -RNA-DNA hybrids bound to the filters counted. Similar hybridisations were carried out with bands A obtained in other experiments and with the analogous *imm* RNA isolated as the fraction excluded from the G-75 Sephadex column. ^{32}P -RNA counts hybridising to $\phi 80\lambda\text{imm}\lambda$ were plotted at the *bio30-7* or *bio124* endpoint position on the abscissa, depending on which of these two λbio l-strand DNAs was used for the prehybridisation of the extracted RNA before its fractionation. The *imm434* and *imm21* endpoint positions refer to the hybridisation values obtained with $\phi 80\lambda\text{imm}434$ and $\phi 80\lambda\text{imm}21$, respectively. After subtracting the $\phi 80$ or $\phi 80\text{hy}42$ background values, all hybridisation values were normalised to the $\phi 80\lambda\text{imm}434$ value taken as 10 units (see ordinate). □, ◇, Induction of the E486($\lambda\text{cI}857\text{cro}^-$) lysogen; RNA prehybridised to $\lambda\text{bio}30-7$ (band A, Fig. 3, in duplicate). ▽, ▴, Induction of the E486($\lambda\text{cI}857\text{cro}^-$) lysogen; RNA prehybridised to $\lambda\text{bio}124$ (band A, not shown, in triplicate). ○, ●, Infection of the E486 host with *cro* $^-$ and *cro* $^+$ $\lambda\text{cI}857$ phage multiplicity of infection 8) respectively; RNA labelled for 0–5 min after infection at 42 °C and prehybridised to $\lambda\text{bio}30-7$ (band A, not shown). ■, Induction of the E486($\lambda\text{cI}857\text{cro}^-$) lysogen; RNA prehybridised to $\lambda\text{bio}124$ (fraction excluded from G-75 Sephadex column with 0.1 M ammonium acetate, pH 4.5 as solvent). ○, Average of all results with λcro^- ($\lambda\text{cI}857\text{cro}27$). Abscissa corresponds to the physical map of phage λ (see Fig. 1 and ref. 16).



Preliminary fingerprints of that portion of band A RNA which hybridises to $\phi 80\lambda\text{imm}21$ DNA indicate that this 5'-proximal sequence is identical to that of *oop* RNA (refs 20 and 21), (to be published elsewhere).

oop leader sequence and its dual role: p_o compared with p_{re} promoter

The present study indicates that *oop* RNA can serve as a leader sequence and can be extended into the immunity transcript. This concept of termination-anti-termination (or attenuation) was known in λ transcription (see, for example, ref. 6) and later found in other operons^{23,24}. Such a mechanism was postulated as one of the alternatives when *oop* RNA was first characterised^{6,13,14}. Our results now make it unnecessary to invoke the presence of a p_{re} promoter to explain the cis-dominant effect of the *c42* and other *cY* mutations in the γ region^{2,3,25,26} (Fig. 1). The *cY* mutations might represent either some polar blocks or inactivation of one of the sites at which the *cII* and *cIII* products act as anti-terminators and enable the extension of *oop* RNA into the *imm* transcript. The latter explanation seems less likely, since the *cII* product is still required for suppression of the *cY* defect by the *sar* mutation¹⁰. This subject is now being studied. Note that the p_o promoter region and the *cIII* product are in common for phages λ and $\lambda\text{imm}21$ (hy1)²⁷, but the *cII* products differ. If the anti-terminating action of the *cII* protein depends on DNA sequence specificity, the first divergence in the sequence between λ and $\lambda\text{imm}21$ is at about 60 nucleotide pairs from the 5' end of *oop* (M. Rosenberg, personal communication). It might be relevant that recognition for the N product, as defined by the *nutL* mutations, is over 30 nucleotide pairs downstream from the p_L promoter of λ (J. S. Salstrom and W. Szybalski, cited in ref. 17).

The simplest model consistent with the present and previous data is that *oop* RNA may serve a dual role, both as a primer for λ DNA replication¹⁵ and as a primer or leader for the

immunity establishment transcription. In conditions which presumably favour early *cro* expression, only short species of *oop* RNA would be synthesised, and this abundance of a primer for the initiation of DNA synthesis would channel the phage into the lytic cycle, which requires extensive λ DNA replication. Alternatively, probably when *cro* gene expression is slightly delayed or depressed, the availability of the *cII* and *cIII* products would favour the elongation of the *oop* RNA into the *imm* transcript. This would lead to a burst of repressor synthesis and consequently channel the phage into the lysogenic pathway and establishment of the prophage state. It is interesting that the recently described *sar* mutation, which maps in the *oop* region, seems in certain conditions to favour the lysogenic pathway while depressing DNA synthesis¹⁰.

Although this study has demonstrated new important features of the establishment mode of the immunity transcription, future work is needed to elucidate the function of the γ region and the specific mechanisms by which the *cro*, *cII* and *cIII* products regulate the elongation of the *oop* RNA into the *imm* transcript.

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letters to nature

Sigma Orionis E as a mass-transfer binary system

SIGMA Orionis E, the first He-rich B star discovered^{1,2}, was recently found^{3,4} to have an extremely broad ($\sim 1,800 \text{ km s}^{-1}$), rapidly varying H α -emission feature. From independent spectroscopic, spectrophotometric, and photometric observations on 4 consecutive nights in December 1974, we have shown⁴ that it is a variable, with $P \approx 1.19 \text{ d}$; however, incomplete phase coverage in those data, as well as uncertainty about the nature of the periodicity, has hampered the development of a model to account for the wealth of observed phenomena. Here we briefly communicate results of new continuous $uvby\beta$ photometry carried out during the 11 consecutive nights December 28, 1975–January 7, 1976 (UT), together with some possible interpretations.

The observations were made with a single-channel, refrigerated, pulse-counting, 1P21 photometer on the number 1 0.4-m telescope of the Cerro Tololo Inter-American Observatory. As in the December 1974 observations, CTIO filter set number 3 was used throughout; however, for the new data differential photometric techniques were employed. The comparison star was HR1861, a $uvby\beta$ standard star^{5,6} with colours nearly identical to those of σ Ori E and $\sim 1^\circ$ away. Ten-second integrations were used. An observation, requiring $\sim 5 \text{ min}$ for completion, consisted of the measures: $y, b, v, u, \beta W, 2\beta N$, recentre, and repeat in reverse order, where W and N refer to the wide and narrow H β filter, respectively. The nights were essentially moonless and sky readings were taken regularly throughout. Errors from photon counting statistical effects were $< 0.007 \text{ mag}$ in all observations. Differential extinction corrections were applied to the $uvby\beta$ data with coefficients determined each night, except for 2 nights, greatly shortened by clouds, for which the means of the immediately adjacent nights were adopted. The H β data were reduced using the well known mean slope coefficients for the filters and a nightly determination of the zero point correction based on HR1861.

The resultant light and colour curves, of which those for y , u , and $u-b$ are given in Figs 1a–c, show two distinct minima. By comparison of the depths, particularly in u , we conclude that the minimum near phase $\phi \approx 0.63$ (hereafter called primary minimum) is the same as that observed⁴ in December 1974. A primary minimum occurred in the new observations⁷ at JD 2442778.819, which combines with the December 1974 data to yield an improved estimate of the period: $1.19080 \pm 0.00005 \text{ d}$. This value is in excellent agreement with one possible period, 1.19086 d, found from a comparison of the December 1973–January 1974 spectroscopic³ and the December 1974 spectro-

photometric⁴ H α -emission data; this agreement suggests that the period may be reasonably stable over a 2–3 yr interval.

Among the characteristics of the rather peculiar light curves, we point out the following: (1) The secondary minimum follows the primary by $0.43P$ (12.4 h), and the widths of both are $\sim 3.0 \text{ h}$ at half-depth. (2) The amplitudes of the minima are considerably greater in the ultraviolet, and the depths in y , b , v , and u of the primary (0.07, 0.06, 0.05, and 0.15 mag) and secondary (0.04, 0.04, 0.03, and 0.12 mag) minima vary non-monotonically with wavelength. The amplitudes of the $u-b$ colour minima are, however, similar. (3) The minima are asymmetrical, with the ingress into secondary minimum more gradual (by a factor of ~ 2) than the other transitions. (4) The light level outside the minima is not constant but seems to decline continuously between successive secondary minima, although the v light level is more irregular. With respect to December 1974 the mean magnitude levels at $0.8P$ have remained nearly constant in y and b , but the u level is brighter by $\sim 0.04 \text{ mag}$ in 1975–76; this observation suggests that secular changes with a colour dependence similar to that of the periodic ones may occur. (5) The H β phase diagram shows behaviour strongly reminiscent of that of W_{bs} (H α) in Fig. 4 of Walborn and Hesser⁴, which suggests that weak, variable emission is present at H β . Comparably detailed observations of more cycles are required to ascertain the permanence of the foregoing features in the light and colour curves, but correspondences between the December 1974 and 1975–76 data indicate that some of them are reasonably stable.

The general appearance of the light curves of σ Ori E, and that of the H α profile when the emission is strong, are strikingly similar to observed properties of the dwarf nova system U Geminorum^{8,9}. These properties are interpreted in terms of a mass-transfer binary in which a collapsed (white dwarf) component is surrounded by a rapidly rotating accretion disk containing an ultraviolet-bright spot with energy provided by the impinging stream of gas from the other star^{10,11}. It is evident that a similar model could account for most of the principal characteristics of the light, colour and spectrum variations in σ Ori E. Particular reference is made to the illustration and discussion by Lin¹². The primary minimum occurs when the hot spot is occulted by the B star, whereas the secondary minimum is caused by occultation of the spot by the optically thick disk itself. Since the spot is located on the following side of the disk, the secondary minimum will follow the primary by $< 0.5P$, as observed. Moreover, since the line-emitting region is downstream from the hot spot in the disk, the principal H α -emission maximum should follow inferior conjunction of the disk and have $V > R$, again as observed⁴. We note, however, that this model requires synchronous

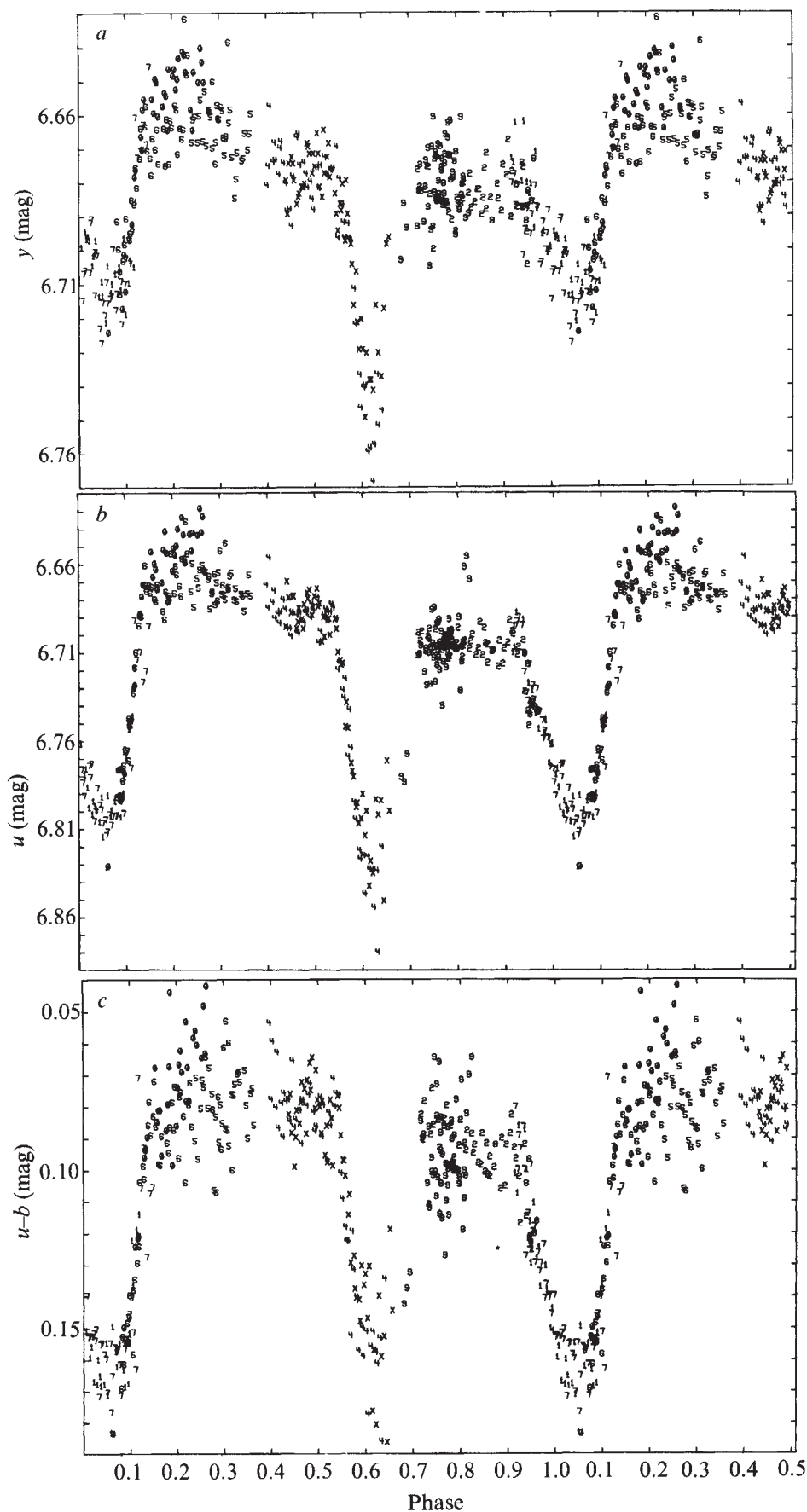


Fig. 1 *a-c*: y , u , and $u-b$ light and colour curves for σ Ori E from the 1975-76 data, with 1975 December 28.0 = JD 2442774.5 as zero phase and a period, 1.19082 d, that differs insignificantly from the finally adopted value, 1.19080 d. The symbols serve to distinguish data from different nights: 0-9 correspond to December 28-January 6 and $\times$ to January 7. Because of occasional high humidity during the observations, it is uncertain if some fine details of the light curves (for example, the tendency for night 0 (28 December) to lie somewhat brighter than those of nights 5 or 6 at $\phi \approx 0.2$) arise from stellar variations or to slightly inaccurate differential extinction corrections. The $u-b$ colour is (normally) a reliable temperature indicator for B stars.

rotation and a stable, inhomogeneous surface composition for the B star to explain the behaviour of the He line^{4,13}.

Bolton's¹⁴ upper limit to the radial velocity variation of σ Ori E implies that any companion must have a mass $\lesssim 0.1M_{\odot}$ for inclinations $> 45^{\circ}$. Thus the secondary would be an M dwarf or a collapsed object. The latter alternative would be in agreement with the above model, as well as providing a ready explanation for the helium enhancement (or hydrogen deficiency) at the surface of σ Ori E in terms of a prior episode of mass transfer from an originally more massive companion. Since σ Ori E is located in a Trapezium-like system containing a 0.9.5-V component², the mass of the original primary in the mass-transfer binary picture would have been $\gtrsim 20M_{\odot}$.

If this model is correct, high-frequency optical monitoring of σ Ori E might provide additional information; only one such study has been made at CTIO, before the development of the present working picture, and hence at a rather low sampling frequency. Beginning at 1974 December 6.084 UT, σ Ori E was monitored at H α for 6 h with the 3-channel photometer at the 0.9-m telescope. A 20/80% neutral beamsplitter, 2 FW 130 (S20) photocells, and KPNO filters 210 ($\Delta\lambda = 159 \text{ \AA}$) and 71 ($\Delta\lambda = 54 \text{ \AA}$) were used for 2-s integrations. A total of 8,192 integrations was processed with power spectral techniques¹⁶; no peak > 0.0013 mag was found in the period range $4.0 < P < 1,640$ s for the sampled phases.

While this paper was in preparation, an alternative but related interpretation of the light curves of σ Ori E occurred to one of us (N.R.W.) after reading the new results on Algol¹⁶. The alternative model follows from interpreting the two minima in σ Ori E as components of a single 'W-shaped' eclipse; indeed, the resulting ingress-egress symmetries strongly support the correctness of such an interpretation. In this case one is led to consider two ultraviolet-bright spots, most probably located at the equilateral Lagrangian points of the binary system, with the leading spot somewhat the brighter. 'Mid-eclipse' occurs at $\phi \simeq 0.83$, with both Lagrangian points visible. The mass ratio condition for the existence of stable, equilateral Lagrangian points implies an upper limit to the secondary mass of the same order as that resulting from the radial velocity observations. If a plausible model with $M_1 = 8M_{\odot}$, $M_2 = 0.1M_{\odot}$, and centre-to-centre separation of two primary radii is assumed, the resulting geometry is strikingly simple, and many features of the light and colour curves are explained in a straightforward manner.

The implications of this model of σ Ori E for current theories of stellar evolution in massive binaries are sufficiently significant to justify extensive further observations. Most critically needed are high-accuracy radial velocities throughout the full 1.19 d cycle, preferably with simultaneous photoelectric monitoring. In addition, polarimetric, X-ray and radio observations of both moderate- and high-time resolution may provide key information for the evaluation of the proposed working model.

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Evidence for a radioactive decay hypothesis for supernova luminosity

VAN HISE¹ has observed that the light curve for the Type I supernova 1937c is well represented by a sum of two exponentials with half lives which are ~ 0.75 the half lives of ^{56}Ni and ^{56}Co in the β -decay chain $^{56}\text{Ni} \rightarrow ^{56}\text{Co} \rightarrow ^{56}\text{Fe}$. Colgate and McKee² had previously suggested this chain as a source of supernova luminosity, but the hypothesis was not widely accepted because of the variability of the decay rates of supernova light curves and because the interstellar medium would be overabundant in heavy elements. This letter presents observational evidence supporting a model of Leventhal and McCall³ which overcomes these difficulties.

According to this model, a Type I supernova produces a white dwarf containing $\sim 0.2M_{\odot}$ of ^{56}Ni . At terrestrial densities the decays of ^{56}Ni and ^{56}Co occur by capture of an inner shell electron 100 and 80% of the time, respectively, but in the interior of a white dwarf, the bare nuclei move through a Fermi sea of electrons, and the higher electron densities thus encountered would produce an enhancement of the decay rates. The supernova luminosity is given by

$$L(t) = A_1 \exp(-t/T_1) + A_2 \exp(-t/T_2) \quad (1)$$

where T_1 and T_2 are the half lives of the steep, earlier and the flat, later portions of the light curve, and A_1 and A_2 are parameters whose values depend on the initial amount of ^{56}Ni , the

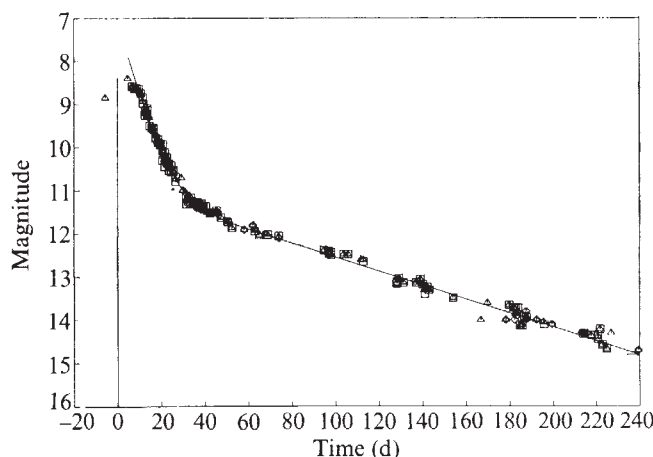


Fig. 1 Sum of exponentials fit to the light curve of SN1937c. The vertical line at $t = 0$ represents the estimate m_0 of peak apparent magnitude. $\square$, Baade and Zwicky⁸; $\triangle$, Parenago¹⁰; $\diamond$, Deutsch¹¹. For the values of the parameters of the fit see Table 1.

energy releases per decay of ^{56}Ni and ^{56}Co , and the values of T_1 and T_2 . If the model is correct, T_1 should not exceed the terrestrial half life of ^{56}Ni (8.8 d), T_2 should not exceed that of ^{56}Co (112 d), and the two should be related approximately by

$$\frac{T_1}{8.8} = \frac{T_2}{112}; T_2 = 12.7 T_1 \quad (2)$$

More precisely, according to Leventhal and McCall³, at

Table 1 Results of the sum of exponentials fits

Supernova	Galaxy	$\frac{A_1}{L_0} \pm \sigma \frac{A_1}{L_0}$	$T_1 \pm \sigma(T_1)$	$\frac{A_2}{L_0} \pm \sigma \frac{A_2}{L_0}$	$T_2 \pm \sigma(T_2)$
1937c	IC4182	3.18 ± 0.13	6.47 ± 0.16	0.0980 ± 0.0088	67.2 ± 6.8
1937d	NGC1003	1.36 ± 0.11	9.26 ± 1.09	0.0708 ± 0.0471	102.1 ± 92.4
1939a	NGC4636	3.07 ± 1.45	4.12 ± 1.34	0.159 ± 0.073	59.3 ± 33.7
1954a	NGC4214	1.52 ± 0.14	4.14 ± 0.32	0.142 ± 0.014	55.6 ± 5.4
1954b	NGC5668	2.19 ± 0.56	5.70 ± 1.16	0.0964 ± 0.0513	75.9 ± 54.9
1956a	NGC3992	11.9 ± 6.5	4.59 ± 0.70	0.0896 ± 0.0302	87.7 ± 38.4
1957a	NGC2841	1.82 ± 0.25	4.55 ± 0.43	0.130 ± 0.021	47.4 ± 8.4
1961p	Anon.	1.64 ± 0.07	10.3 ± 0.7	0.0979 ± 0.0259	112.3 ± 40.5
1962j	NGC6835	3.14 ± 1.12	7.48 ± 1.34	0.111 ± 0.048	114.8 ± 81.4
1962l	NGC1073	3.39 ± 0.78	5.21 ± 0.77	0.181 ± 0.064	68.1 ± 34.6
1963p	NGC1084	5.44 ± 1.19	4.96 ± 0.44	0.0684 ± 0.0217	61.2 ± 20.5
1964l	NGC3938	2.65 ± 0.49	5.96 ± 0.46	0.0671 ± 0.0081	120.4 ± 22.9
1966j	NGC3198	4.44 ± 2.18	7.11 ± 1.01	0.0766 ± 0.0108	112.3 ± 22.4
1966n	Anon.	1.34 ± 0.10	8.38 ± 1.36	0.0917 ± 0.0384	85.4 ± 44.7
1967c	NGC3389	2.17 ± 0.19	8.17 ± 1.00	0.129 ± 0.101	47.9 ± 30.4

high matter densities the ^{56}Ni and ^{56}Co decay rates (d^{-1}) are given by

$$R_{\text{Ni}} = \frac{1}{8.8} \left(\frac{\bar{\rho}_{\text{av}}}{9.7 \times 10^{28}} \right) (1 + 0.61 \times 10^{10} \bar{\rho}_{\text{av}}^{-1/3})$$

$$R_{\text{Co}} = \frac{1}{558} + \frac{1}{139.4} \left(\frac{\bar{\rho}_{\text{av}}}{8.6 \times 10^{28}} \right) (1 + 0.60 \times 10^{10} \bar{\rho}_{\text{av}}^{-1/3})$$

where $\bar{\rho}_{\text{av}}$ is the electron density (cm^{-3}), 8.8 and 112 d are the terrestrial 1/e lives of ^{56}Ni and ^{56}Co , 9.7×10^{28} and $8.6 \times 10^{28} \text{ cm}^{-3}$ are the electron densities at Ni and Co nuclei in the terrestrial environment, and $1/558 \text{ d}^{-1}$ represents the fraction (20% on Earth) of Co decays which involve positron emission. It is assumed here that the positron emission rate is unaffected by high densities. At low densities, electron binding occurs, so the effective decay rates can be calculated by the interpolation equations

$$T_1 = \left[\left(\frac{1}{8.8} \right)^2 + R_{\text{Ni}}^2 \right]^{-1/2}$$

$$T_2 = \left[\left(\frac{1}{112} \right)^2 + R_{\text{Co}}^2 \right]^{-1/2}$$

which define a curve well approximated by the straight line, equation (2).

Rust⁴ has recently collected 37 Type I light curves from the literature and used techniques developed by Pskovskii^{5,6} to reduce them to a common photometric system (the m_{pg} system). Fifteen of the curves were complete enough to give reasonable fits using a sum of two exponentials. The others did not extend far enough to define the more slowly decaying exponential.

The light curves consisted of measurements of apparent magnitude $m(t)$ versus time. Apparent magnitude is related to luminosity by

$$\frac{L(t)}{L_0} = 10^{-0.4[m(t) - m_0]} \quad (3)$$

where L_0 and m_0 are the luminosity and apparent magnitude at maximum brightness. Taking the moment of peak brightness as the zero-point of time and combining equations (1) and (3) gives

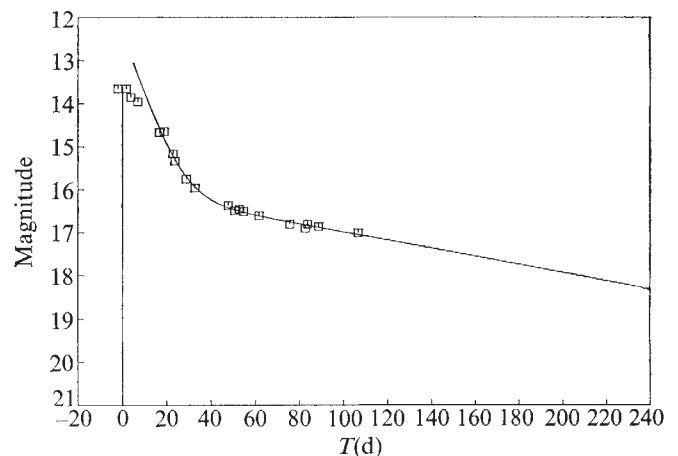
$$10^{-0.4[m(t) - m_0]} = \frac{A_1}{L_0} \exp(-t/T_1) + \frac{A_2}{L_0} \exp(-t/T_2) \quad (4)$$

The values of T_1 , T_2 , (A_1/L_0) and (A_2/L_0) were determined for each light curve by nonlinear least squares fitting of this equation beginning a few days after peak brightness to assure that only the decay portion contributed to the fits. The method used was a damped Gauss-Newton iteration.

The results of the fits are given in Table 1. The standard deviations were estimated from the covariance matrix but should be regarded only as approximations (see ch. 7, ref. 7). The values were checked by repeating the fits using the separable parameter algorithm of Golub and Pereyra⁸.

For all light curves in the sample the fitted curves represented the data points well and gave low values for the sum of squares of residuals, but only if the data were very complete did the fits specify the parameters with high certainty. This is illustrated in Figs 1 and 2 which represent one of the best and one of the worst fits. In both cases the curves accurately represent the data, but for SN1962j, $\sigma(T_2)$ is very large relative to T_2 . The condition that the s.d. of both 1/e lives be less than the parameter values themselves provided a criterion for rejecting incomplete light curves from the sample.

Figure 3 is a plot of T_2 against T_1 with the error bars representing 1 s.d. The vertical and horizontal dashed lines represent the upper limits imposed by the terrestrial values. Only one point lies >1 s.d. beyond these limits, that point being ~ 2 s.d. out. Furthermore, there is a definite tendency for the points to lie along the predicted line $T_2 = 12.7 T_1$ which is shown as a diagonal. Although the scatter is large, the correlation coefficient is $r = 0.529$, implying, at the 97.5% level of significance, that the data follow a non-random relationship. The average values of $\sigma(T_1)/T_1$ and $\sigma(T_2)/T_2$ are 0.13 and 0.43, a fact which invalidates both a standard regression of T_2 on T_1 , which would

Fig. 2 Sum of exponentials fit to the light curve of SN1962j. Data points are taken from Bertola¹².

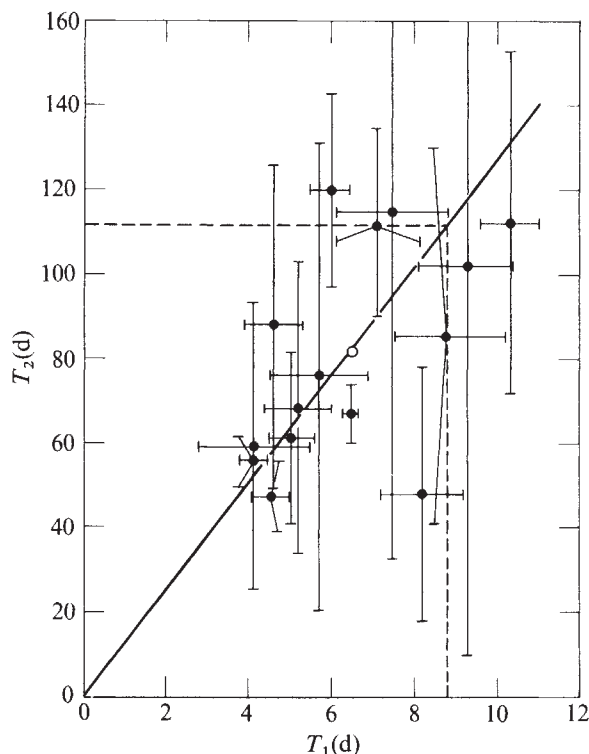


Fig. 3 Plot of the fitting parameters T_2 against T_1 . The error bars represent the estimated s.d. but should not be interpreted as the major axes of error ellipsoids because the errors in T_1 and T_2 are correlated in each case.

assume the errors in T_1 are insignificant, and a major-axis regression, which would assume approximately equal errors in T_1 and T_2 . It is, however, possible to use the computer generated T_1, T_2 variance matrices to obtain a maximum likelihood estimate of the best linear representation of the data (see ch. 4, ref. 7). The likelihood function can also be used to construct confidence regions in the space of the two parameters, that is, slope and intercept (ch. 7, ref. 7). When this procedure was applied to the data the point defined by equation (2), that is, slope = 12.7, intercept = 0, was found to lie well inside the 50% contour. This shows that the data are consistent with the predicted relation. A more dramatic measure of this consistency is furnished by the average values of T_1 and T_2 which are

$$\begin{aligned}\overline{T_1} &= 6.46 \pm 1.98 \\ \overline{T_2} &= 81.2 \pm 25.7\end{aligned}$$

where the indicated errors are the s.d. The point represented by these values is shown in Fig. 3 as an open circle lying almost exactly on the predicted line. These results constitute significant evidence supporting the $^{56}\text{Ni} \rightarrow ^{56}\text{Co} \rightarrow ^{56}\text{Fe}$ decay hypothesis. Furthermore, if the cold white dwarf model is correct, the fact that no T_2 is < 47 d implies an upper mass limit of $\sim 0.4 M_\odot$ for white dwarfs produced.

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Orientation of pulsar magnetic dipole moments

THE angle between the magnetic dipole moment and the spin of a pulsar is an important parameter in studies of the pulse emission. In this letter, arguments based on the properties of the weak interaction between elementary particles and on the superfluidity of the core show that the torque associated with the rotating dipole moment is free to cause alignment with the spin only in pulsars older than the Crab, and possibly the Vela pulsar. On the basis that the sine of the angle follows an exponential law with a time constant of $\sim 10^6$ yr, the observed distribution of radio pulse widths can be explained by the group of models in which emission occurs from regions near the polar caps rather than the light cylinder.

The high electrical conductivity¹ of the interior matter implies that the magnetic field of a pulsar should be fixed with respect to the body axes of the star. The principal moments, and the torque acting on the star, determine the orientation, as a function of time, of the magnetic dipole moment relative to the spin. The torque can be expressed as the sum of three components

$$\mathbf{N} = \mathbf{N}_1 + \mathbf{N}_2 + \mathbf{N}_3$$

where $\mathbf{N}_1$ and $\mathbf{N}_2$ represent the electromagnetic torque and are, respectively, perpendicular and parallel to the magnetic dipole moment $\mathbf{p}$. The component $\mathbf{N}_1$ can be approximated by the vacuum torque for the rotating external dipole field, and $\mathbf{N}_2$ represents the torque resulting from current flow between the corotating magnetosphere and the polar caps of the star. The component $\mathbf{N}_3$ represents the dissipative torque, which causes the axis of greatest principal moment to align with the spin. Owing to the temperature- and therefore time-dependence of $\mathbf{N}_3$, the angle ψ between spin and dipole moment is predicted for pulsars with the properties: (1) a mass such that there is no solid core; (2) an internal toroidal magnetic field with symmetry axis approximately colinear with $\mathbf{p}$, to have the following time variation. At a time $t_1 \lesssim 10^3$ yr, ψ becomes approximately equal to $\pi/2$ and remains constant until $t_2 \gtrsim 10^4$ yr. For $t > t_2$, $\psi \rightarrow 0$ with a time constant determined by $\mathbf{N}_1$, $\mathbf{N}_2$ and by the values of ψ and of the period P at $t = t_2$. This form of $\psi(t)$ is consistent with the existence of a strong inter-pulse in the Crab pulsar and, if the slowing of the pulsar rotation is determined mainly by $\mathbf{N}_1$, provides a simple explanation of the secular variation of magnetic dipole moment postulated by Lyne, Ritchings and Smith².

This prediction of $\psi(t)$ depends only on general features of superfluidity and of the non-leptonic weak interaction. Accurate knowledge of energy gaps or of the equation of state is not required.

At certain radii in the interior, neutrons, protons and Σ^- hyperons are expected to be in equilibrium. The protons³ form an isotropic BCS superfluid with energy gap Δ . For such densities, the neutrons⁴ may have a small anisotropic energy gap. If the proton energy gap alone is considered, the relaxation time for the non-leptonic weak interaction $n+n \rightleftharpoons p+\Sigma^-$ is proportional⁵ to $(\beta\Delta)^{-5/2} \exp(\beta\Delta)$ in the limit $\beta\Delta \gg 1$, where $\beta^{-1} = k_B T$. If the spin is not parallel with the axis of greatest principal moment, the matter in the interior is subject to periodic pressure fluctuations having the period of the precession which must occur. As the pulsar cools, the relaxation time must increase until of the order of the precession period so

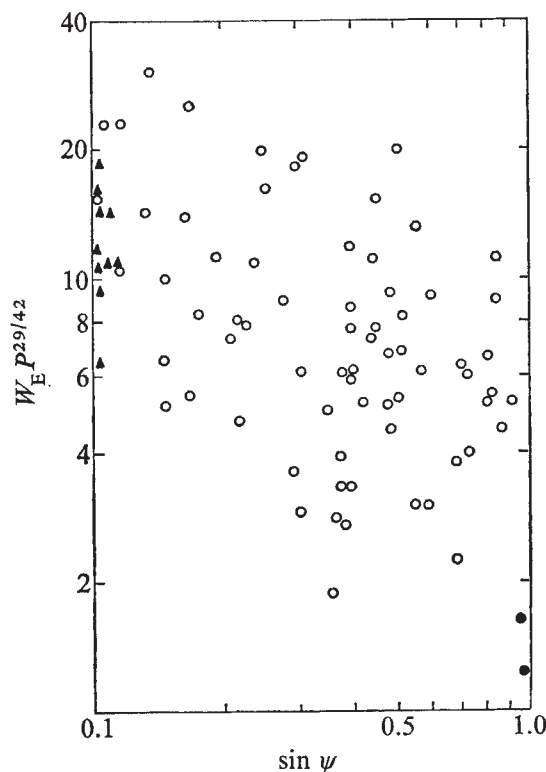


Fig. 1 The distribution of $W_E P^{29/42}$ and $\sin \psi$ is shown for 82 pulsars, for $\tau_D = 10^6$ yr. The integrated pulse widths W_E , expressed in degrees, are those at 408 MHz listed by Lyne *et al.*². The 10 pulsars with $\sin \psi < 0.1$ are shown separately as solid triangles on the left-hand side of the diagram. The Crab and Vela pulsars are likely to have $t < t_2$ and are represented by the two solid circles at the lower right-hand side.

that the coefficient of bulk or second viscosity is extremely large. In the Euler equations, the effect of the viscosity is expressed as a large torque N_3 which causes the axis of greatest principal moment to become parallel with the spin at a time $t_1 \lesssim 10^8$ yr. The effect of a small neutron energy gap would be merely to reduce the value of t_1 . Further cooling of the pulsar interior causes the viscosity and therefore N_3 to become negligibly small at times $\sim 10^4$ yr. A more detailed discussion is given elsewhere (P. B. Jones, unpublished).

The distortion caused by the elasticity of the solid outer shell has an axis of symmetry which moves with respect to fixed body axes in such a way as to be approximately parallel to the spin direction. This motion is the result of plastic deformation which occurs for $t \lesssim t_1$ because the temperature is then of the order of 10^8 K and because the rotational surface oblateness greatly exceeds the likely critical strain. Thus the axis of greatest principal moment is determined by the toroidal internal magnetic field and is approximately perpendicular to $\mathbf{p}$.

For $t > t_2$, and $N_1 \gg N_2$ and N_3 , $P \sec \psi$ is a constant of motion and $\sin \psi$ decreases according to an exponential law with a mean time $\tau_D = P^2 \sec^2 \psi (P\dot{P})^{-1}_{t=t_2}$. Owing to this alignment of the dipole moment, its component perpendicular to the spin has the time dependence postulated by Lyne *et al.*². Plausible values of P , the period derivative $\dot{P}$, and ψ at $t_2 \simeq 10^4$ yr are quite consistent with $\tau_D \simeq 10^6$ yr given by Lyne *et al.*, whereas if the dissipative torque were neglected, $t_2 = 0$ and the predicted values of τ_D would be too small^{6,7} to be consistent with the observed pulsar population.

In one group of pulse emission models⁸⁻¹⁰, the microwave radiation is produced tangentially to their velocity by charged particles moving along field lines leaving the polar caps of the star and penetrating the light cylinder. The integrated pulse width is a function of the angle ψ and of the angular size of the

emission cone, which is dependent on the period. For the most recent model¹⁰ in this group, $W_E P^{29/42}$ should be proportional to $(\sin \psi)^{-1}$, apart from variations in the intersection of the emission cone by the line of sight. The expression for W_E given by a second group of models¹¹, those involving relativistic beaming, is very dependent on the velocity of the emitting region and no simple prediction seems possible.

Values of $\sin \psi$ have been determined from the $\dot{P}$ measurements of Lyne *et al.*² for a universal $\tau_D = 10^6$ yr. The distribution of $W_E P^{29/42}$ and $\sin \psi$ shown in Fig. 1, a band of gradient -1 , supports the polar cap group of models and the hypothesis that most pulsar magnetic dipole moments are aligning with the spins in a mean time $\sim 10^6$ yr. If the points shown as open circles were normally distributed about a straight line, the gradient of the line would be -0.99 ± 0.15 .

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Ocean bottom seismic measurements on the East Pacific Rise and Rivera Fracture Zone

We describe preliminary results of a seismic experiment using ocean bottom seismometer capsules (OBS) in three separate tripartite arrays. The arrays were located on the central topographic high of the East Pacific Rise between the Rivera and Tamayo Fracture Zones (8 d in position), at the junction of the ridgecrest and the Rivera fracture zone (12 d), and on the Rivera fracture zone well away from the ridgecrest (9 d). Accurate locations and depths could be computed for events sufficiently close to each array, and showed that the main transform fault lay within the central trough. OBS capsules have been successfully used for refraction work^{1,2} (also B. Lewis and C. Lister, unpublished) and microseismicity studies³, but we report here the first use of arrays on a spreading centre-transform fault system for event locations. Other questions of importance to seismology can also be answered from these data.

The capsules⁴ contain a 1-Hz vertical seismometer and triggered digital recording system. The capsule itself falls freely to the ocean bottom. When triggered acoustically or after a predetermined time, it is released and floats to the surface. An acoustic system facilitates accurate location of the capsule to within several metres relative to a surface ship.

Figure 1 shows sites and epicentre locations for a number of events recorded at arrays 2 and 3. Array 1 (the ridge array) is not shown. Figure 2 shows a sample of seismograms from array 3. At array 2, 105, 680, and 155 events were recorded on the 3 capsules, respectively. Of these, 67, 57, and 37 were recorded simultaneously by each of the three pair combinations of capsules. Thirty-one earthquakes were recorded simultaneously by all three capsules. Some of the events recorded (particularly those on LY), are spurious triggers caused by ground noise. Many, however, are earthquakes too small to trigger all capsules. All events recorded simultaneously on two or more capsules are believed to be earthquakes. At array

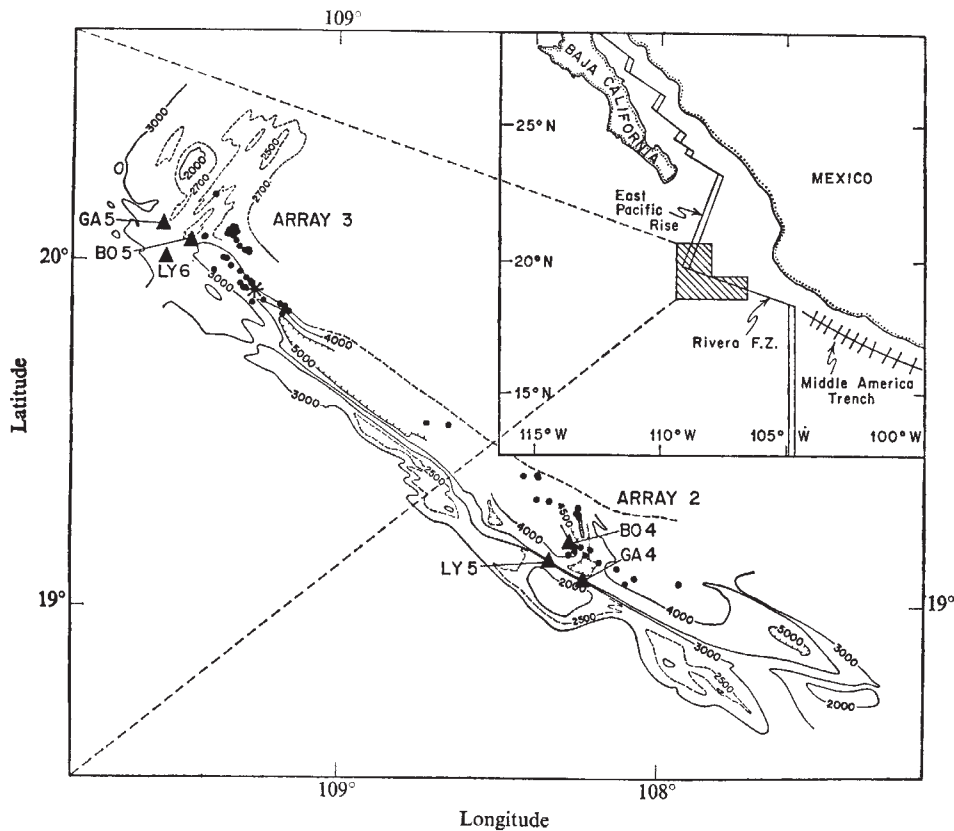


Fig. 1 Capsule sites and bathymetry (m). All of the well determined epicentres recorded at array 2 are shown. A representative sample of events recorded at array 3 are plotted. The asterisk shows the location of the swarm sequence.

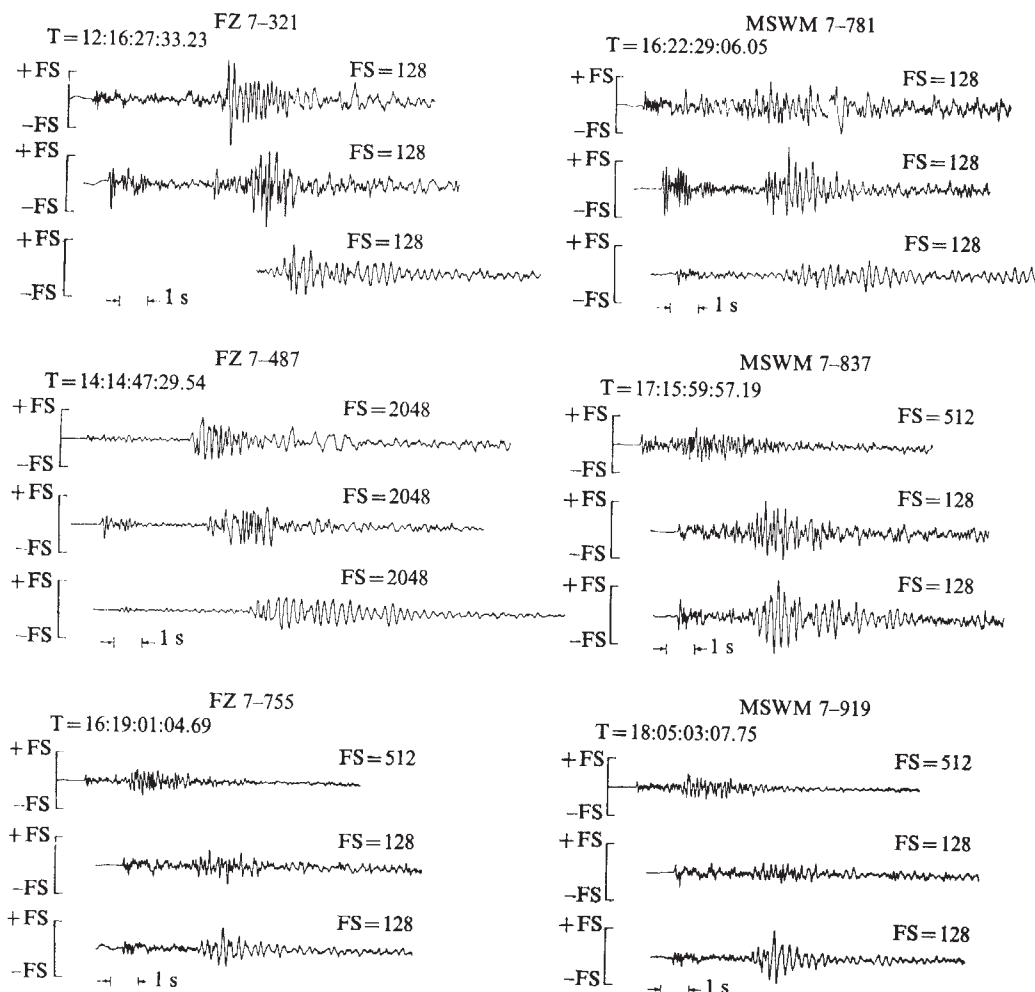


Fig. 2 This shows several seismograms recorded at array 3. Each group of 3 was recorded at BO5 (top), LY6 (middle), and GA5 (bottom). Events 7-755, 7-783, and 7-919 are from the small swarm sequence due east of the array. The others are down the fracture zone towards the asterisk. At 5 Hz, the seismometer response in counts μm^{-1} is 2.4×10^3 . FS on the plot is in counts.

number 3, 254, 304, and 409 events, respectively, were recorded by the capsules. The pair combinations of capsules recorded 196, 213, and 309 events simultaneously. Of these, 183 were recorded on all three capsules. During this period, a swarm of earthquakes with a $M=4.3$, $M=4.3$, and $M=4.8$ sequence, and numerous smaller events, was recorded. The three larger events were recorded on World-Wide Network stations.

The locations of capsules BO and LY were determined by acoustic ranging, and triangulation from the ship, in conjunction with satellite fixes and/or fixes to a moored radar buoy. We estimate the accuracy of these locations to be within 100 m. Capsule GA's transponder failed, so we obtained the best possible estimate of ship position and drift before, during and after the drop. We estimate the location accuracy of GA to be within 400 m.

Epiceentre locations and depths were calculated using a half-space location programme written by Ray Buland at IGPP. The locations shown in Fig. 1 were calculated with $V_P=6.5$ and $V_P/V_S=\sqrt{3}$. Seismic refraction results from the East Pacific Rise⁵ indicate that this velocity is reached within the top 2 km of crust. For sources a few km deep, as these are believed to be, the half-space model is probably a good approximation as long as the events are near or within the array. Locations of events close to the array were found to be relatively insensitive to changes in velocity. All reasonable values for V_P gave epicentres and source depths which varied by at most 1 or 2 km. The locations of the more distant events varied by several km, but our basic conclusions are not affected by the uncertainty of velocity structure.

The tectonics of the area are shown schematically in the inset to Fig. 1 along with the bathymetry of the northern part of the Rivera Fracture Zone. A deep, steep-sided trough is flanked by ridges on both sides. The topography is generally extremely rugged. To the south-east, beyond the area shown, the trough disappears and the fracture zone continues as a single ridge. This change in character coincides with a change in strike, the topography trending more in an east-west direction.

Three immediate conclusions may be drawn from the pattern of seismicity near array 2:

(1) The general trend of epicentres is along the central trough. This suggests that transform faulting is taking place mainly inside this feature, rather than along the valley walls.

(2) There is a definite offset in the line of epicentres near the array. It is unlikely that this offset is an artefact of location errors. Location uncertainties make it impossible to draw any similar conclusions about the more distant events.

(3) A few of the events have epicentres very close to one of the capsules. This allows well-constrained source depths to be calculated. All reliable source depths were shallower than 10 km below the sea floor, suggesting 10 km as a lower limit for the thickness of the brittle zone on this area.

With array 3, we tried to cover the junction of the rise and fracture zone. There seem to be no epicentres located within the array. The epicentres shown in Fig. 1 for this array are some of the more reliable locations (that is, with the smallest travel-time residuals). We have not yet located all the events in this area, but the epicentres shown are probably a fairly good sample.

As in the central part of the fracture zone, the main trend of epicentres is along the rift. A few km from the ridge there is a second line of activity, parallel to the first. This might indicate an offset of the main transform fault, or possibly a subsidiary fault reflecting greater complexity of the faulting as the junction is approached.

The exact location of the spreading centre is uncertain, but presumably coincides with the topographic high of the rise. It seems that, as would be expected, the seismicity does not extend beyond this. We also note that there is a swarm-like cluster of events near the junction, east of the array. These results are similar to those of Reid and Macdonald⁶ on the Mid-Atlantic Ridge. Since all the events located lie outside the array, little reliance can be placed on source depth determinations. It

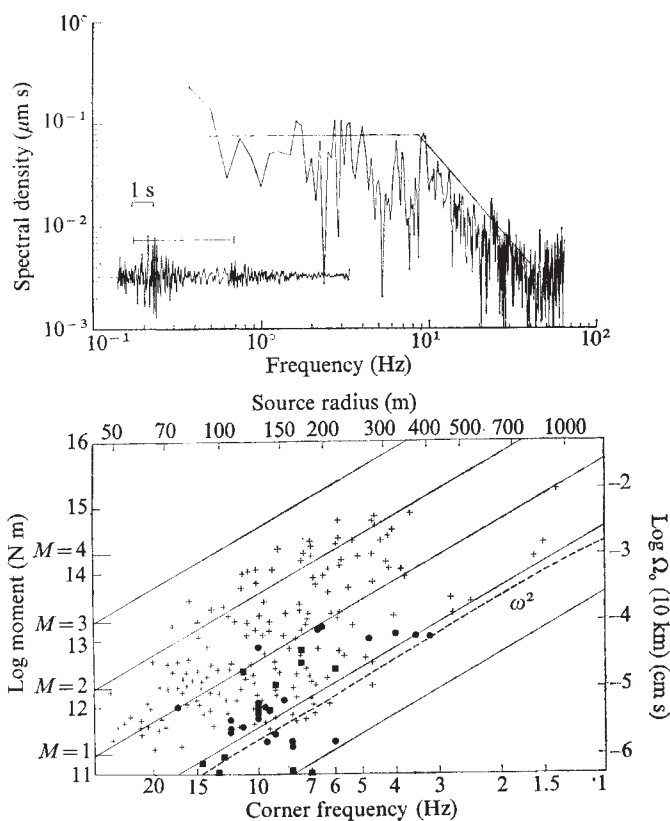


Fig. 3 Source parameters of 35 events recorded at Arrays 2 and 3. The upper portion of the figure is a ground motion spectrum of the S-wave of one of the events (Event 22). The line over the seismogram indicates the portion of the signal analysed. The source parameters from the aftershocks of the San Fernando earthquake of February 9, 1971 are shown (+) as a comparison to the oceanic data (■, Array 2; ●, Array 1). The diagonal lines give a logarithmic pressure scale, the top line gives the 10^6 -Pa region (1 Pa = 10^{-5} bar), and each subsequent line gives a factor of 10 smaller a pressure, down to 10^4 Pa at the bottom.

appears, however, that the depths of the nearest events are shallow, probably < 5 km.

S-wave spectra of 35 events of various sizes from arrays 2 and 3 have been computed. The spectra have been corrected for instrument response and an assumed Q of 250. This is a reasonable value of Q for S-waves on continental crust⁷. The moments and corner frequencies of nearby events are also relatively insensitive to the value of Q chosen. The instrument response was computed using measured seismometer and amplifier parameters. Figure 3 shows the spectrum of event 22 measured at capsule position BO 4 (Fig. 1). The rise at high frequencies is caused by the Q correction, which has the effect of dividing the background noise by a very small number. Also in Fig. 3 is a plot of the seismic moments against the corner frequencies of the 35 events studied. Although the results must be considered preliminary until more analysis is carried out, the stress drops estimated using the theory of Brune^{8,9} do not reach values as high as those for aftershocks of the San Fernando earthquake of February 9, 1971, also plotted in Fig. 3 (ref. 7).

We conclude that epicentres of micro-earthquakes on the Rivera Fracture Zone indicate that the main transform fault lies inside the central trough. There is evidence that at least one offset occurs in this fault. One interpretation of this would be that this offset represents a minor spreading centre similar to, but on a smaller scale than, those in the Gulf of California. A small component of spreading on this section of the fracture zone would be consistent with the interpretation of the central trough as a rift feature. Source depths suggest a brittle zone ~10 km thick. S-wave spectra of 35 events suggest a lower upper-bound for stress drop than found for the San Fernando earthquake of February 9, 1971.

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Further rubidium–strontium age and isotope evidence for the nature of the late Archaean plutonic event in West Greenland

THE gneisses of the Godthaabsfjord region of southern West Greenland have been grouped by McGregor¹ into an older and a younger set, respectively termed the Amitsoq and Nûk gneisses. They are separated by dykes (the Ameralik dykes), metavolcanic and metasedimentary rocks (the Malene supracrustals) and stratiform meta-anorthosites. The Amitsoq gneisses have been dated at ~3,700–3,800 Myr (refs 2–4). Pankhurst *et al.*⁵ reported a Rb–Sr whole rock isochron age of $3,040 \pm 50$ Myr and an initial $^{87}\text{Sr}/^{86}\text{Sr}$ ratio of 0.7026 ± 0.0004 on a suite of Nûk gneisses from Bjørneøen, some 25-km northeast of Godthaab (Fig. 1). The isochron age was interpreted as the age of emplacement of the precursors of the Nûk gneisses, while the low initial ratio suggested that these precursors could not have been derived by partial or complete melting of the older Amitsoq gneisses. Several geologists have expressed disagreement with this interpretation^{6,7}. In particular, Chadwick *et al.*⁷ disagreed with our conclusion that the Nûk gneisses represent essentially juvenile sialic crust formed in West Greenland ~2,800–3,000 Myr ago. We here present further evidence to support our interpretation.

A large collection of Nûk gneisses was made by us in 1973 from the area between Buksefjord and Sermilik (Fig. 1, also Table 1, Numbers 4, 5, 6). Some of these samples are from

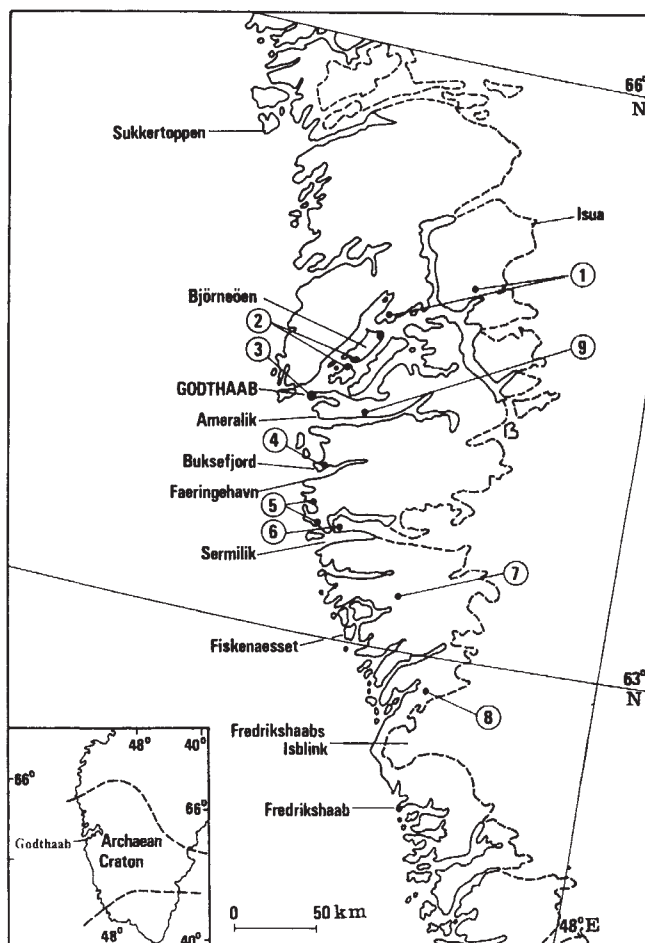


Fig. 1 Sketch map of the areas sampled. The numbers are the same as in the first column of Table 1.

localities where Chadwick *et al.*⁷ believed they had unequivocal evidence for remobilisation of Amitsoq gneiss. A more recent, preliminary, report of the geology of the area has been given by Chadwick *et al.*⁸.

Further suites of Nûk-type gneiss samples were donated for this study by D. Bridgwater and V. R. McGregor from the Godthaabsfjord region (Fig. 1, also Table 1, Numbers 1, 3) and by J. S. Myers from Fiskenaesset (Fig. 1, also Table 1, Number 7).

We summarise in Table 1 Rb–Sr whole rock isochron ages and initial $^{87}\text{Sr}/^{86}\text{Sr}$ ratios obtained on a total of 96 samples of Nûk-type gneisses grouped into different areas (Fig. 1). We also present whole rock data for the post-tectonic Qôrqt Granite (Fig. 1, also Table 1, Number 9), the last major rock-forming

Table 1 Summary of Rb–Sr age and isotope results for Nûk-type gneisses and Qôrqt granite

Area (see Fig. 1)	No. of Samples	Mean Rb (p.p.m.)	Mean Sr (p.p.m.)	R^*	Age (Myr)†	Initial $^{87}\text{Sr}/^{86}\text{Sr}$	$F^\ddagger$
1. Head of Godthaabsfjord	11	82	494	0.48	$2,850 \pm 60$	0.7017 ± 0.0002	1.5
2. Bjørneøen§	14	64	415	0.45	$2,930 \pm 70$	0.7023 ± 0.0003	2.5
3. Godthaab	11	44	584	0.22	$2,780 \pm 150$	0.7026 ± 0.0005	3.0
4. Buksefjorden	21	60	278	0.63	$2,870 \pm 60$	0.7015 ± 0.0003	3.0
5. Faeringehavn	8	74	248	0.86	$2,790 \pm 60$	0.7031 ± 0.0005	2.0
6. Sermilik	20	72	270	0.77	$2,780 \pm 30$	0.7020 ± 0.0002	1.5
7. Fiskenaesset (Majorqap qáva)	11	86	488	0.51	$2,880 \pm 50$	0.7016 ± 0.0003	2.5
8. Fredrikshaabs Isblink††	13	47	276	0.50	$2,660 \pm 120$	0.7032 ± 0.0010	<1
9. Qôrqt Granite, Ameralik	7	215	133	4.8	$2,520 \pm 90$	0.709 ± 0.007	1.0

* $R = ^{87}\text{Rb}/^{86}\text{Sr}$ calculated from mean Rb and Sr.

†Decay constant for ^{87}Rb is $1.39 \times 10^{-11} \text{ yr}^{-1}$.

‡ F = Error factor required to achieve least squares fit (that is M.S.W.D. ≤ 2). Errors are quoted at 2σ , after taking F into account.

§From Pankhurst *et al.*⁵, reanalysed.

††From Pidgeon and Hoppood¹⁸.

event in the Godthaabsfjord region¹. (Individual analytical data for these 103 samples are not reported here, but may be obtained on request from the authors.) Full details of chemical and mass-spectrometric techniques have been described elsewhere⁹. Rb/Sr ratios were determined by a precise X-ray fluorescence (XRF) technique described by Pankhurst and O'Nions¹⁰.

New analyses on the 14 samples from Björneöen reported by Pankhurst *et al.*⁵ (Table 1, Number 3) yield slightly revised age and initial $^{87}\text{Sr}/^{86}\text{Sr}$ values of $2,930 \pm 70$ Myr and 0.7023 ± 0.0003 , mainly from recalibration of the XRF method using USGS standard rocks¹⁰. These are, however, still within the error of the published values, quoted earlier. The ages reported in Table 1 for groups 1 to 7 range from 2,780 to 2,930 Myr, whilst the initial $^{87}\text{Sr}/^{86}\text{Sr}$ ratios range from 0.7015 to 0.7031. The errors within each range do not completely overlap, so that small, significant differences appear to exist, as discussed later. Nevertheless, our principal observation is that the grouping is remarkable for its overall coherence in age and initial ratio over the huge area sampled (Fig. 1).

Figure 2 is a composite Rb–Sr whole rock plot for all samples from groups 1–7 reported in Table 1. For comparison, the published values for the Amitsoq gneisses are also plotted^{2,4}. The plot demonstrates the lack of overlap between the Nûk and Amitsoq linear arrays and also shows that both gneiss groups exhibit a similar range of Rb/Sr ratios. This is not surprising, considering the close overall petrological and geochemical similarity between the comparable variety of rock types (for example, granites, granodiorites, tonalites and diorites) which make up both the Nûk and Amitsoq gneisses. This in itself argues against formation of the Nûk gneisses by reworking of Amitsoq gneisses, since this would be expected to result in a general increase of Rb relative to Sr in the remobilised material.

Several samples from the Sermilik area (Table 1, Number 6) are at granulite grade, whereas all other samples in the present study are presumed to be at amphibolite grade. Clearly, metamorphic grade has had no effect on the Rb–Sr age or initial $^{87}\text{Sr}/^{86}\text{Sr}$ of the rocks. This accords with the view that the parent magmas of the Nûk gneisses were emplaced during an igneous event which was broadly contemporaneous with high-grade metamorphism and gneissification. This conclusion has been advanced specifically for the Ilivertalik granite on the south side of Sermilik, to which some of the present samples, which were all collected on the north side of Sermilik, may indeed belong, and which has yielded a U–Pb zircon age of $2,835 \pm 10$ Myr (ref. 11). Moreover, the mean Rb–Sr age of $\sim 2,850$ Myr reported here for Nûk gneisses agrees within analytical error with the mean $^{207}\text{Pb}/^{206}\text{Pb}$ whole rock age of granulite facies gneisses and associated anorthosites from the Nordland–Sukkertoppen and Fiskenaeset areas, respectively north and south of Godthaab (Fig. 1)¹². The Pb/Pb age was interpreted as the probable age of the granulite facies metamorphism, whilst the actual Pb isotope ratios suggested that the gneisses were not reworked Amitsoq gneisses but represented an addition of crustal material from the upper mantle source regions within the period $\sim 2,800$ – $3,000$ Myr ago.

Figure 3 shows averaged Rb–Sr growth lines for the major rock units so far analysed from West Greenland, plotted in relation to a hypothetically linear upper mantle growth line. Two groups of much younger gneisses from South Greenland $\sim 1,700$ – $1,800$ Myr old¹³, are also plotted for comparison.

Consideration of Fig. 3 supports our earlier conclusion that the Nûk gneiss precursors were not derived by partial melting and mobilisation of Amitsoq gneisses, since the average growth lines of the latter are far too steep. By 2,850 Myr ago, the average $^{87}\text{Sr}/^{86}\text{Sr}$ ratios of Amitsoq gneisses would have been ~ 0.705 – 0.715 . This argument is strongly reinforced by the similarity in range of Rb/Sr ratios between the Nûk and Amitsoq gneisses already noted above (Fig. 2).

We conclude that the “irrefutable” identification of Amitsoq gneisses by Chadwick *et al.*^{7,8} in the Buksefjorden–Sermilik area is contradicted by the dissimilarity of the present data with the established Rb–Sr chronology in the Godthaab region. In

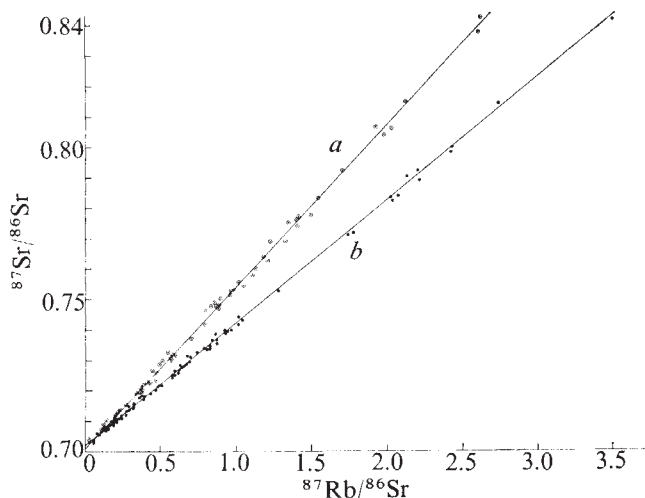
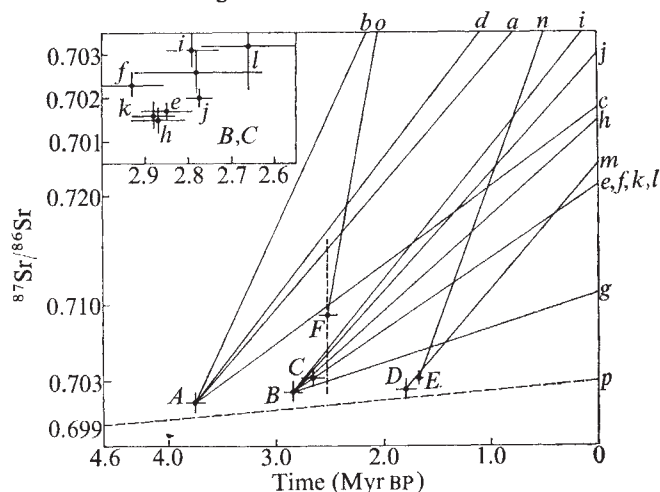


Fig. 2 Rb–Sr whole rock isochron plot for *a*, Amitsoq gneisses^{2,4}, with 3,700-Myr reference line; *b*, Nûk-type gneisses, Groups 1 to 7, Table 1, with 2,850-Myr reference line. Three very high Rb/Sr points have been omitted from each line.

this connection, four whole-rock samples from just north of Sermilik, cut by amphibolite fragments considered as correlative with the Ameralik dykes, are included in Table 1, Number 6. They cannot be distinguished from the main body of data and separately yield an isochron age of $2,760 \pm 80$ Myr and an initial $^{87}\text{Sr}/^{86}\text{Sr}$ ratio of 0.7020 ± 0.0004 .

At the present time there is very little evidence for Archaean rocks with high $^{87}\text{Sr}/^{86}\text{Sr}$ ratios. Hurst *et al.*¹⁴ report an age and initial $^{87}\text{Sr}/^{86}\text{Sr}$ ratio of $3,120 \pm 60$ Myr and 0.7064 ± 0.0012 for a group of late “Undifferentiated gneisses” from Saglek, Labrador, whilst Barton¹⁵ has quoted a value of 0.7044 ± 0.00010 for gneiss from Hebron, Labrador dated at $\sim 3,600$ Myr, although in the latter case we believe there has been a serious underestimation of the error. Barton quotes errors calculated by two different methods but, for no stated reason, uses the lowest error value. Little is known of the chemistry of the Saglek “Undifferentiated gneisses” and the possibility that there has been redistribution of ^{87}Sr in these rocks on the scale of sampling

Fig. 3 Average $^{87}\text{Sr}/^{86}\text{Sr}$ growth lines for: *A*, Amitsoq gneisses^{2,4} from *a*, Narssaq; *b*, Qilangarsuit; *c*, Praestefjord; *d*, Isua; *B*, Nûk-type gneisses (Table 1) from *e*, head of Godthaabsfjord; *f*, Björneöen; *g*, Godthaab; *h*, Buksefjorden; *i*, Faeringehavn; *j*, Sermilik; *k*, Majorqap qáva, Fiskenaeset; *C*, *l*, Fredrikshaab Isblink¹⁸; *D*, *E*, Ketilidian gneisses, South Greenland¹³; *m*, Group 1; *n*, Group 2; *F*, Qôrqt Granite, Ameralik. The line *p* represents a hypothetically linear upper mantle growth line. The inset is a magnification of points *B* and *C* for the Nûk-type gneisses listed in Table 1.



following a previous crustal history cannot be discounted. Such a process would mean that partial melting and intrusion of magma need not necessarily have occurred at $3,120 \pm 60$ Myr ago. The only unequivocal case known to the authors in which Rb–Sr systems demonstrate “isotopic reworking” of Archaean crust is a Rb–Sr whole-rock isochron of $2,300 \pm 150$ Myr with an initial $^{87}\text{Sr}/^{86}\text{Sr}$ ratio of 0.7126 ± 0.0011 for gneiss from Vesterålen, Norway¹⁶. Pb isotopes in the same rocks preserve an age of $\sim 3,500$ Myr, however, so that in this case it is unlikely that “isotopic reworking” involved segregation of a partial melt.

The Qôrqt granite of West Greenland (Table 1, Number 9) gives a whole-rock isochron age of $2,520 \pm 90$ Myr and initial $^{87}\text{Sr}/^{86}\text{Sr}$ of 0.709 ± 0.007 , in good agreement with a previously published mineral isochron for a related pagmatite¹⁷. The high Rb/Sr ratios of the analysed samples have so far precluded more precise determination of the initial $^{87}\text{Sr}/^{86}\text{Sr}$ ratio and this therefore remains ambiguous in terms of its petrogenetic implications. In view of the more extreme granitic aspect of the Qôrqt granite (predominantly quartz and K-feldspar with few mafic minerals) compared to the earlier gneisses it might well be derived by remobilisation of old crustal material.

The small but statistically significant range of age and initial $^{87}\text{Sr}/^{86}\text{Sr}$ ratios of the Nûk type gneisses in West Greenland (see Table 1 and inset, Fig. 3) may genuinely reflect small differences in the age of emplacement and pre-history of the parent magmas. We believe that a plutonic episode of such magnitude comprises complex, multistage processes lasting for perhaps 100–200 Myr. It is, however, also possible that the gneisses have variously suffered partial redistribution of radiogenic ^{87}Sr during the course of this episode, so that it is not possible to be absolutely sure that each of the isochrons in Table 1 records the specific age of emplacement of any one suite of cogenetic igneous rocks. The fact that none of the best fit lines are perfect isochrons (for which $F \leq 1$ in Table 1), and that there is a faint suggestion of inverse correlation between isochron age and initial $^{87}\text{Sr}/^{86}\text{Sr}$ ratio within the 2,900–2,650-Myr interval, emphasises this doubt, although the latter observation is rather heavily biased by the results of Pidgeon and Hopgood¹⁸ from much further south at Frederikshaabs Isblink (Fig. 1, also Table 1, Number 8).

Our major conclusion remains that the igneous precursors of the Nûk gneisses throughout the area sampled represent a massive addition of new calc-alkaline material to the Archaean continental crust of West Greenland. These magmas separated from a low Rb/Sr source region, such as upper mantle, not more than 200 or 300 Myr at the most before the measured isochron ages of Table 1. Geochemical differentiation, magmatism and metamorphism all occurred during the same major plutonic episode^{19,20}. There is as yet no isotopic evidence for the involvement of Amitsoq gneisses in the formation of the Nûk gneiss precursors.

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Characterisation of monounsaturated fatty acids from an estuarine sediment

PREVIOUS investigations of fatty acids in marine sediments have primarily been concerned with the concentrations of the individual acids rather than their chemical configuration^{1–4}. Brown and Swidler⁵ have demonstrated the ability of clay to catalyse the conversion of oleic acid (18:1 Δ^9 *cis*) to elaidic acid (18:1 Δ^9 *trans*) accompanied by extensive rearrangement of the double bond position. (18:1 = normal carbon chain length: number of double bonds). As most biologically produced olefinic acids are believed to be in the *cis* configuration, diagenetic changes in the sediment may alter the *cis* isomers to the more stable *trans* isomers, as well as cause migration of the double bond. Consequently, investigations of the monounsaturated fatty acid *cis/trans* ratio and position of the double bonds, with depth in the sediment, may yield significant information on diagenetic pathways of these acids. We have determined the presence of small amounts of palmitelaidic acid (16:1 Δ^9 *trans*) and elaidic acid (18:1 Δ^9 *trans*), as well as other positional isomers of these *trans* acids, in a Recent estuarine sediment. In addition, we have also confirmed that the major monounsaturated fatty acids are palmitoleic acid (16:1 Δ^9 *cis*) and oleic acid (18:1 Δ^9 *cis*). We give here the methods for isolating these acids and confirming their structures, and their geochemical significance is discussed.

The general collection and analytical procedures have previously been reported in detail^{1–3}. Surface sediment (upper 8–10 cm) was collected from station D in Narragansett Bay, Rhode Island². The sediment was saponified (under reflux) for 2 h using 2:1 (v/v) 0.5 N KOH in 90% methanol–benzene, filtered through a Whatman No. 1 filter, and the filtrate transferred to a separatory funnel. After addition of distilled water to give two phases, the non-saponifiable fraction was removed and backwashed with pH 10 distilled water. The resulting aqueous phase and the initial saponifiable fraction were then both acidified to pH 2 and extracted three times with petroleum ether. The combined ether phases were evaporated to near dryness at room temperature under reduced pressure using a rotary evaporator. Subsamples were then taken for each analysis.

To subsamples I and II were added 17:1 Δ^{10} *cis* or 17:1 Δ^{10} *trans* fatty acid internal standards. Each subsample was then methylated using BF₃–methanol and the methyl esters isolated by thin-layer chromatography (TLC)¹. The methyl esters were further separated on AgNO₃–TLC to isolate both *cis* and *trans* monounsaturated esters^{6,7}.

The *cis* and *trans* fractions were individually analysed by flame ionisation gas–liquid chromatography (GLC) using a Hewlett–Packard model 5750 gas chromatograph. The resulting chromatogram for the *trans* methyl esters is shown in Fig. 1. The proper configurational assignments were made on the basis of relative retention times and coinjection with authentic standards on a stainless steel column (6m $\times$ 2.2mm inside diameter) containing GP–15% SP–2340 on 100/120 Chromosorb P, AW-DMCS (Supelco), resolution⁸ = 0.83 for 16:1 Δ^9 *cis*

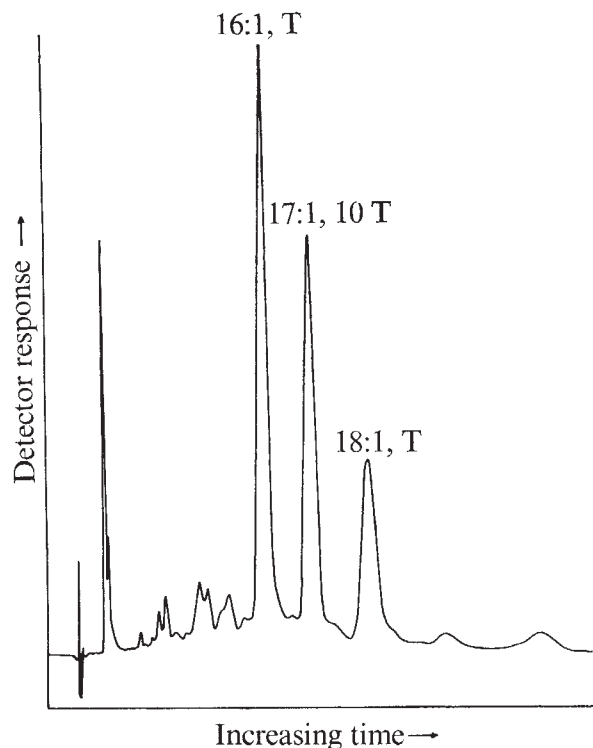


Fig. 1 Gas chromatogram of 16:1 and 18:1 *trans* methyl esters, in presence of 17:1 Δ^{10} *trans* methyl ester internal standard, recovered from station D surface sediment.

and *trans*. Each sample was also hydrogenated and co-injected with standard saturated esters

To determine the quantitative amount of each acid, the areas of the peaks of the naturally occurring acids (before and after hydrogenation) were compared with the areas of the internal standard. (No natural 17:1 *cis* or *trans* was detected in the sediments.) The GLC analysis of I and II indicated the quantities of fatty acids in the sediment to be as follows (μg acid per g dry weight of sediment): for 16:1 *cis*, $17.1 \mu\text{g g}^{-1}$, for 16:1 *trans*, $1.7 \mu\text{g g}^{-1}$, for 18:1 *cis*, $9.6 \mu\text{g g}^{-1}$, and for 18:1 *trans*, $0.8 \mu\text{g g}^{-1}$. Analytical uncertainty was found to be $\pm 10\%$ or less. Thus in both cases, the *cis* isomer accounted for $\sim 92\%$ and the *trans* isomer $\sim 8\%$ of the total 16:1 and 18:1 acids.

To investigate the possibility of the *trans* isomer being generated from the *cis* isomer during the saponification, methylation, or TLC procedures, a series of blanks was carried through the entire analysis. Each blank consisted of a pre-extracted sediment plus various combinations of 16:1, 17:1 and 18:1 *cis* standard acids. No *trans* acids were generated during this procedure

The configurational assignments were verified by infrared spectroscopy and combined gas chromatography-mass spectrometry (GC-MS) analyses. Two separate subsamples, III and IV, were processed by the above methods without internal

standards. Portions of the purified *cis* and *trans* methyl esters were each analysed by GC-MS (Finnigan 1015C). The resulting GC-MS spectra for these esters were compared with spectra for authentic standards and agreed well in all cases. The GC-MS spectrum for the *trans* methyl ester of the eighteen carbon fatty acid is shown in Fig. 2. This spectrum shows the molecular ion peak at m/e 296 (M^+). Some characteristic fragmentation peaks are m/e 265 ($M^+ - 31$), m/e 264 ($M^+ - 32$), m/e 222 ($M^+ - 74$), and m/e 74 (McLafferty rearrangement)^{9,10}.

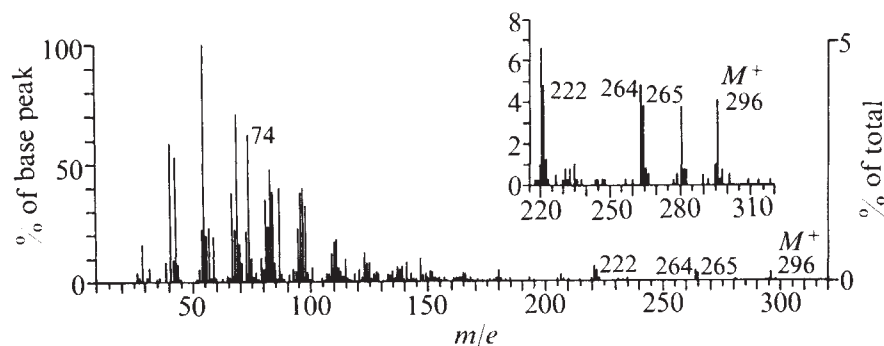
Separate portions of III and IV were analysed by infrared spectroscopy (Perkin Elmer model 521) and compared with authentic standard infrared spectra. The infrared spectrum of the *trans* methyl esters is presented in Fig. 3 and shows the characteristic *trans* absorption at 965 cm^{-1} ($10.3 \mu\text{m}$). The infrared spectrum of the *cis* methyl esters showed both the absence of the *trans* band at 965 cm^{-1} and the presence of a stronger absorption in the region of $3,015 \text{ cm}^{-1}$ ($3.32 \mu\text{m}$). The infrared and GC-MS spectra together with the GLC analyses confirm the presence of both 16:1 and 18:1 *cis* and *trans* acids.

Portions of III and IV were oxidised using KMnO_4 in a modification of Downing and Greene's¹¹ procedure to determine the position of the double bond. The sixteen and eighteen carbon *cis* oxidation products yielded primarily the C_7 and C_9 methyl esters and the C_9 dimethyl ester, indicating the double bond to be predominantly in the 9-10 position for both fatty acids. The ratios of the dimethyl esters recovered indicate that $\sim 62\%$ of the double bonds in the *cis* isomers are in the 9-10 position, 19% are in the 11-12 position, and 19% are in other possible positions from C_3 to C_8 , and C_{10} .

Analysis of the sixteen and eighteen carbon *trans* oxidation products again yielded primarily the C_9 dimethyl ester and the C_7 and C_9 methyl esters, indicating the double bonds in both *trans* isomers to be predominantly in the 9-10 position. The ratios of the dimethyl esters recovered from the oxidation of the *trans* isomers indicate that $\sim 70\%$ of the double bonds are in the 9-10 position, 23% are in the 11-12 position, and 7% are in other positions as above. Within the accuracy of the method, there is no significant difference between the positions of the double bonds in the *cis* and *trans* isomers.

The three most common monounsaturated fatty acids found in organisms are oleic acid, palmitoleic acid, and vaccenic acid (18:1 Δ^{11} *cis*)¹². It appears that the major monounsaturated acids present in the examined surface sediment are the 16:1 Δ^9 , 18:1 Δ^9 and 18:1 Δ^{11} . These are present as $\sim 92\%$ in the *cis* configuration and 8% in the *trans* configuration. Since the double bonds are primarily in two positions, the 9-10 and 11-12 positions, this would seem to indicate that the *cis* isomers present in this surface sediment result from a direct biological input. The *trans* isomers result from either a direct input or from microbial modification of the *cis* isomers in the sediments. The *trans* isomers apparently do not result from the clay catalysed isomerisation of the *cis* isomer. According to Brown and Swidler⁵ the clay catalysed isomerisation of the *cis* octadecenoic acid yields a mixture of the *trans* isomers with the double bond ranging from the 2-17 positions, and the 9-10 *trans* isomer making up only 23% of the total.

Fig. 2 GC-MS spectrum of 18:1 *trans* methyl ester recovered from station D surface sediment; no internal standard added (m/e 220-310 expanded in upper right).



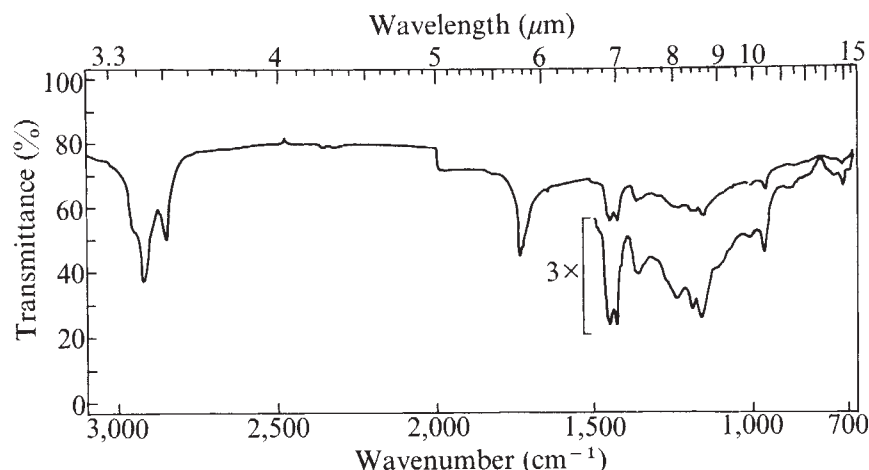


Fig. 3 Infrared spectrum of 16:1 and 18:1 *trans* methyl esters recovered from station D surface sediment; no internal standard added.

Although the absolute quantities of the monounsaturated fatty acids normally decrease with depth in the sediment², secondary effects may result from clay catalysis. As depth increases, the clay catalysed conversion of the *cis* to *trans* isomers may occur until an equilibrium mixture of *cis* and *trans* results (for the 18:1 acid, the equilibrium mixture is ~ 33% *cis*, 67% *trans*^{5,13}). As the *cis* isomer is isomerised to the *trans* isomer, it may also result in a migration of the double bond throughout the molecule. Thus with increasing depth in the sediments, there exist two potential biogeochemical markers based on monounsaturated fatty acids, the ratio of *cis/trans* and the position of the double bond.

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Evidence for short term atmospheric ¹⁴C variations about 4,000 yr BP

It is well established from radiocarbon measurement of tree rings of known age that the natural ¹⁴C level in the Earth's atmosphere has undergone a gradual change of more than 10% over the past 7,000 yr (see, for example, ref. 1). During this time there have probably also been periods of irregular fluctuation spanning a few hundred years (the so-called Suess 'wiggles'²). The existence, in addition to these effects, of substantial shorter term (~ 10-20 yr) variations possibly correlated with solar

activity is less certain. Farmer and Baxter (ref. 3) found ¹⁴C variations of up to 3% in 10 yr (equivalent to an age error of ±120 yr) over a consecutive sequence of 37 single tree rings taken from mid-nineteenth century wood grown in the Forest of Dean, England; but comparative measurements they made of twentieth century New Zealand wood were inconclusive. Twentieth century wood from Trondheim, Norway, showed a possible 0.6% variation⁴, and twentieth century North American wood showed about 0.3% (ref. 5).

We present here the results of radiocarbon measurements of Red Deer antlers obtained from recent excavations of Neolithic flint mines at Grime's Graves, Norfolk. When taken with the archaeological evidence these results indicate that short term variations similar to those found in wood from the Forest of Dean may also have occurred at an earlier period.

During the later Neolithic in Britain (~2,000 yr b.c. on the basis of radiocarbon dating) flint was mined on a large scale at Grime's Graves. Shafts, typically about 13 m deep and 3-5 m in diameter, were sunk through superficial deposits into the Upper Cretaceous Middle Chalk to reach a more or less continuous 20-cm layer of tabular flint. At the base of the shafts 4-7 horizontal galleries ~ 75 cm-1.5 m high, 3-5 m wide and 5-10 m in length were cut. The antlers that were used for the radiocarbon measurements were used by the miners as levers or 'picks' (Fig. 1) and were found in large numbers amongst the chalk rubble filling the galleries (chalk waste from new galleries

Table 1 Radiocarbon dates for antlers from pit 15

Age estimate (date ± s.d.)*	Δ‰†	Standard deviation of Δ	δ ¹³ C(‰)	Lab. No.
1,786 ± 58	-12.7	4.6	-24.80	(BM-980)
1,870 ± 46	- 6.2	3.6	-24.99	(BM-979)
1,877 ± 45	- 5.6	3.5	-24.16	(BM-973)
1,899 ± 44	- 3.9	3.4	-23.03	(BM-976)
1,915 ± 44	- 2.7	3.4	-25.00	(BM-978)
1,918 ± 56	- 2.5	4.3	-23.32	(BM-1001)
1,932 ± 45	- 1.4	3.5	-21.16	(BM-1002)
1,937 ± 47	- 1.0	3.6	-24.13	(BM-974)
1,940 ± 42	- 0.8	3.2	-23.65	(BM-996)
1,990 ± 41	+ 3.1	3.1	-24.10	(BM-975)
1,999 ± 42	+ 3.7	3.2	-22.46	(BM-1003)
2,010 ± 56	+ 4.6	4.3	-24.87	(BM-997)
2,042 ± 45	+ 7.0	3.4	-23.00	(BM-998)
2,065 ± 61	+ 8.7	4.6	-24.53	(BM-977)
2,072 ± 57	+ 9.3	4.3	-23.25	(BM-1000)

*Dates, expressed in yr b.c. (5,570-yr half life), have been corrected for isotopic fractionation (δ¹³C) relative to the PDB standard. The s.d. was derived from about 15 replicate measurements of each sample (totalling > 10⁴ counts).

†Δ values¹⁰ have been calculated from a mean age of 1,950 yr b.c. See Fig. 2 for the general distribution of these samples within the galleries of pit 15.

was back filled into those from which flint had already been extracted, sealing discarded antlers in position). The antlers were preserved in an environment entirely free from contamination by more recent organic material. To eliminate the possibility of exchange with more ancient carbon only the protein matrix remaining after complete demineralisation with dilute hydrochloric acid was used for the radiocarbon measurements.

Table 1 lists the results of radiocarbon measurements (using the liquid scintillation counting method⁶) on antlers from one of the excavated pit groups (pit 15) originally made to obtain a series of archaeological dates for the mining activity. The measured ages are distributed over a range of some 300 radiocarbon years, with a mean value of 1,950 yr b.c. and a standard deviation for the distribution as a whole of 79 yr. This value is considerably larger than the average standard deviation for the individual age estimates (47 yr). Thus, it is very unlikely (at the 99% confidence level) that each of the samples measured has the same ^{14}C concentration. The data also show a tendency to cluster into an early group (older than 1,990 yr b.c.) and a late group (younger than 1,940 yr b.c.; with one possible outlier at 1,786 yr b.c.) which is related to the find positions of the antlers in the galleries (see Fig. 2). If that were because pit 15 was worked at separate periods the time spread needed to satisfy the data would be of the order of 120 yr, whereas all the available evidence indicates that this pit was actually worked for a single short period which probably did not exceed 10 yr, and may have been much less. One estimate⁷ has suggested that one pit could have been fully exploited in six months. On this basis a reasonable estimate for the exploitation of the entire deep mining area at Grime's Graves (some 400 pits) would be less than 200 yr as groups of adjacent shafts were most probably open contemporaneously.

In support of the idea of rapid exploitation, excavated shafts show little trace of weathering implying that they were open for a short time and were then rapidly infilled artificially with the massive quantities of spoil resulting from the digging of new shafts nearby. Comparison with weathering evidence obtained from studies of artificial earthworks^{8,9} suggests a maximum of 5–10 yr as a cautious overestimate of the length of time for which a shaft was open. Taken altogether this means that the galleries of any one excavated pit complex, whether they radiated from the central pit or from the shafts surrounding it, would most probably all have been cut within a decade at most and the abandoned antlers sealed in place within that time.

Therefore, we must assume that the variation found is attributable to an effect other than real age differences, and is inherent either in the measurement procedures used in our laboratory or in the samples themselves. Taking the first alternative, the samples were measured in small groups interspersed with measurements of other totally unrelated samples, over a period during which our well tried monitoring procedures revealed no abnormalities in the behaviour of the counting system, and we can see no reason to doubt the reliability of the results.

We therefore propose that the variations are most probably attributable to an effect inherent in the samples. Antlers grow

Fig. 1 Deer antler pick.

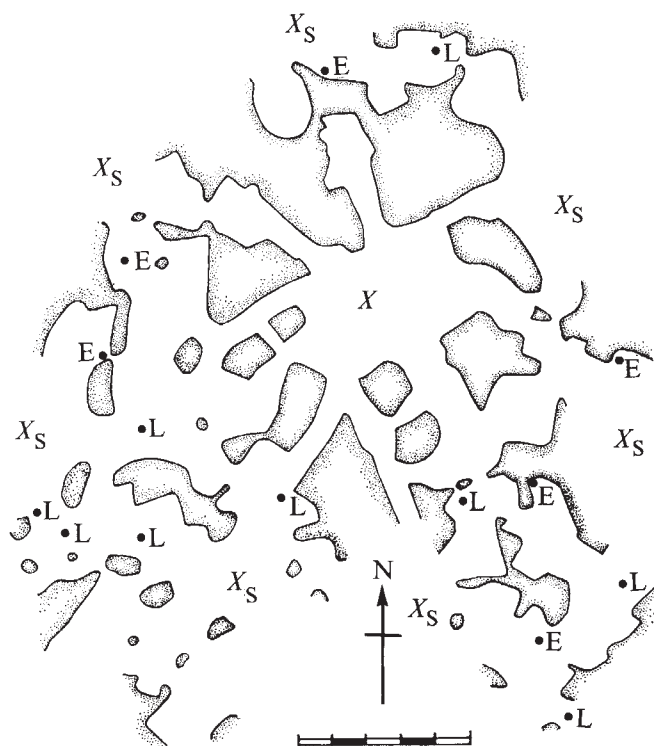


Fig. 2 Diagrammatic plan of the galleries of pit 15 at Grime's Graves, showing original position of each sample. E, L, indicate whether the radiocarbon age estimate obtained falls early or late in the sequence (see text and Table 1). X, Shaft of pit 15; Xs, approximate positions of surrounding shafts. Scale in metres.

and are shed each season, and since they would not be suitable for use as tools unless collected when freshly shed, those accumulated in any one pit complex must form a physical assemblage of short-lived material of a restricted age range. From the number of antlers found (over 70 in pit 15) it is evident also that they were regarded as expendable and were casually discarded when blunted or broken. It is reasonable to assume, therefore, that the ^{14}C activity of the antlers found in a particular gallery closely reflects the atmospheric activity in the summer before that gallery was mined. It is worth noting here that the antlers in the lower left hand corner of Fig. 2 all had a similar activity. The range of ^{14}C activity observed in the antlers from pit 15 as a whole is much larger than can be accounted for unless there were marked year-to-year changes in atmospheric ^{14}C activity. Our results would require a minimum atmospheric ^{14}C variation of the order of 2% over a period of 10 yr or less. In the light of previous findings (refs 3–5) that is not implausible, although the exact cause of the variations remains obscure.

We recognise that our interpretation of these measurements must remain an hypothesis at present but the alternative explanation that the pit was in use over a 120-yr period is contrary to the archaeological evidence. Finally, the stable isotope values ($\delta^{13}\text{C}$, Table 1) do not support the possibility that the observed ^{14}C variations are attributable to dietary factors affecting the deer. Clearly, more work is necessary, and we hope to substantiate our findings by excavating further material from a different pit at Grime's Graves during the coming season.

Our preliminary conclusion is that, although differing fundamentally from tree rings (where either the absolute or relative age is known initially) the special circumstances of the physical association of the antlers provides direct corroborative evidence for the kind of short term ^{14}C variation previously observed in recent tree rings.

More generally, we suggest that although knowledge of the ultimate resolution that radiocarbon dating can achieve is still

inexact, all such assemblages of ancient short-lived material should be regarded as archival stores of geochemical data of great potential value.

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Lead isotope studies of metal sources for ancient Nigerian 'bronzes'

INTEREST in the composition of Nigerian 'bronzes' began in 1897, the year of the British punitive expedition to the city of Benin, when Nigerian metal art was brought to the attention of the western world as a spoil of war. In spite of the dissemination of much of this art to museums, and the subsequent focus of research, important questions concerning the chronological ordering of the pieces and relationships among forms and iconographies remain unanswered.

Elemental analyses of a number of these 'bronzes' (see refs 1–7) reveal that they are actually copper alloys with varying amounts of zinc, lead, tin and other metals. Though patterns of elemental composition can be discerned, statistically well defined measurements are not easily accomplished⁸ and interpretation of the patterns is difficult.

Since the lead content of most pieces is appreciable ($\geq 1\%$ – 12% ; see Table 1), enough, in fact, to suggest that its addition may have been intentional, we have conducted lead isotope analyses. As each lead ore deposit has a set of characteristic

ratios among the isotopes, source distinctions can often be clearly drawn. Small samples in the tens of milligram range have been collected by scraping from approximately 180 'bronzes' from various museums in Nigeria, Europe and the United States. Analyses (see ref. 9) to determine lead isotope ratios have been made of samples from 17 of the more important 'bronzes' (Table 1 and Fig. 1). Only two of the three independent ratios are illustrated in Fig. 1, but all three ratios have been examined with interactive, three-dimensional computer graphics, and compared with isotopic ratios from known lead sources.

The lower panel of Fig. 1 shows a surprisingly strong clustering of the isotopic ratios. All of the Benin pieces have virtually the same isotopic ratios, in spite of a speculated range of production dates spanning approximately from the fifteenth to the nineteenth century. In this same cluster, which we identify as Benin +, pieces identified as 'Owo' (Table 1, B), 'Udo' (J) and 'Lower Niger' (A, G, H and M) are also found. In contrast, the two pieces excavated at Igbo-Ukwu (P and Q), for which there is evidence of a ninth century date² (which has, however, been contested¹⁰), show quite different values for the isotopic ratios. The single piece studied from Ife (O), considered by some¹¹ to be a cultural forerunner of Benin (and identified in Benin oral tradition as the source of Benin metal working knowledge¹²) has intermediate values for the two isotopic ratios shown in Fig. 1. One piece, the 'Lower Niger' ram's head pectoral (H), has two ratios falling in the Benin + cluster and one ratio resembling the Igbo-Ukwu pieces.

Three major conclusions can be drawn. First, the similarity of the isotopic ratios for the Benin + group provides evidence for a single source of lead for the entire time span and variety of locations represented by that group. (The 'Lower Niger' pectoral is a possible exception.) It is unlikely that lead from the Benin + group is a mixture from isotopically different sources since the mixing ratio would have to be nearly constant for all the pieces.

Second, support is lent to the hypothesis that Igbo-Ukwu, Ife and Benin were indeed separate traditions.

Third, the great majority of lead deposits in use today are ruled out as sources, as will be discussed elsewhere.

Several questions are raised, however:

- Why is there such a constancy of lead source throughout the Benin + group in the light of shifting trade patterns within Africa and with Europe.

- What is the nature of the apparent link between the makers of the Benin, 'Udo', 'Owo' and the 'Lower Niger' pieces?

Table 1 Lead isotope ratios in Nigerian 'bronzes'

	$\frac{^{206}\text{Pb}}{^{204}\text{Pb}}$	$\frac{^{208}\text{Pb}}{^{207}\text{Pb}}$	$\frac{^{206}\text{Pb}}{^{208}\text{Pb}}$
A 'Lower Niger' (NML 72.16.26)* Igala bell-head	18.41	1.1705	0.4774
B 'Owo' (Benin?) (I 65.4.1)† Stool	18.41	1.1726	0.4774
C 'Jebba' (NML exhibit) (0.80% Pb; ref. 11) Bowman	18.42	1.1740	0.4785
D Benin (BM 1903.10-22.3)‡ Late Period head	18.47	1.1739	0.4786
E Benin (BM 1963.Af9.1) Early Period head	18.49	1.1757	0.4780
F Benin (BM 97.10-11.1) Early Period head of Queen Mother	18.49	1.1756	0.4780
G 'Lower Niger' (BM 1952.Af11.1) Huntsman	18.50	1.1758	0.4788
H 'Lower Niger' (BM 1930.4-23.1) Ram's head pectoral	18.47	1.1770	0.4839
I Benin (BM 1949.Af46.156) Musician	18.48	1.1763	0.4772
J 'Udo-style' (Benin) (BM 1948.Af9.1) Head	18.43	1.1759	0.4797
K Benin (BM 98.1-15.32) Plaque	18.43	1.1778	0.4789
L Benin (BM 98.1-15.34) Plaque by Master of the Circled Cross	18.43	1.1771	0.4792
M 'Lower Niger' (BM 1956.Af27.224) Head	18.41	1.1771	0.4799
N Benin (BM 97.10-11.1) Early Period head of Queen Mother	18.39	1.1773	0.4788
O Ife (BM 1939. Af34.1) (11.4% Pb; ref. 3) Head	18.51	1.1881	0.4789
P Igbo-Ukwu (NML 39.1.13) (6.4% Pb; ref. 2) Ritual vessel in shape of shell	18.75	1.1912	0.4822
Q Igbo-Ukwu (NML 39.1.2) (4.5%, 9.8% Pb; ref. 2) Bowl	18.90	1.1986	0.4838
Probable errors	± 0.06	± 0.001	± 0.001

*Nigerian National Museum Lagos.

†University of Ife, Institute of African Studies.

‡British Museum, London.

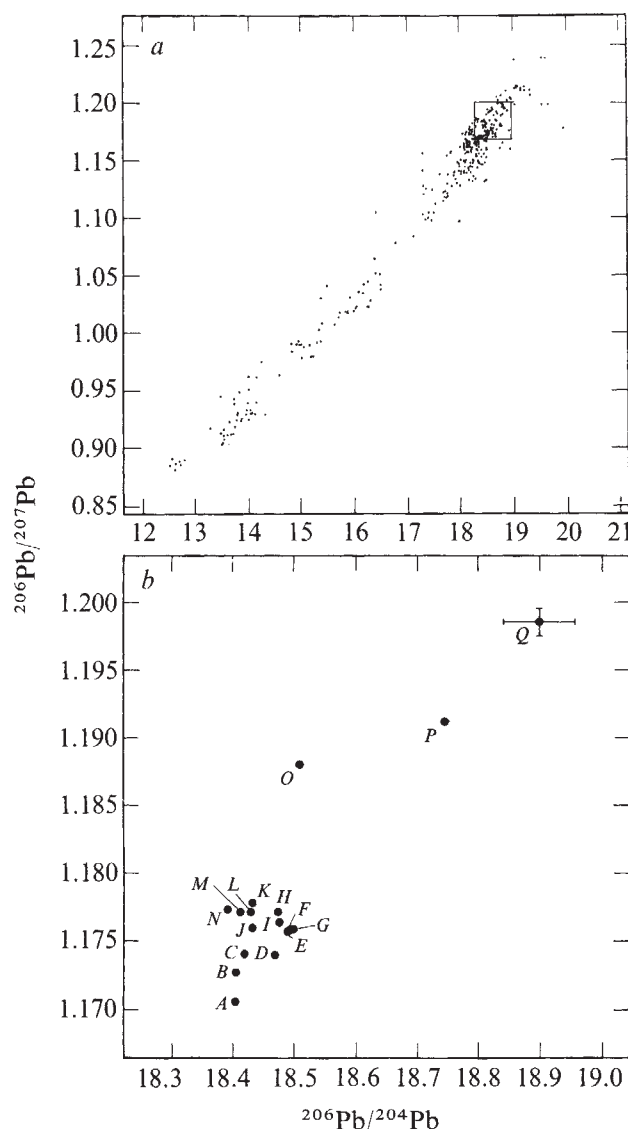


Fig. 1 *a*, Isotopic ratio distribution for geological lead samples from Africa and Europe from the Tables of refs 13–15 (small box corresponds to range shown in *b*); *b*, lead isotope ratios for Nigerian 'bronze' objects listed in Table 1 (probable errors for all measurements are as shown for object Q). A–Q, Objects listed in Table 1.

● Could the Ife piece be a mixture of the sources used by the Igbo–Ukwu culture which may have preceded it, and the Benin culture which may have followed it?

● What were the actual sources of the lead and the patterns of its trade? Were the sources local (Nigerian) or were the raw materials brought from elsewhere in Africa (perhaps on trans-Saharan trade routes) or did they arrive from Europe?

Work is continuing to attempt to answer some of these questions.

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Parental investment, mate desertion and a fallacy

TRIVERS¹ defines parental investment as “any investment by the parent in an individual offspring that increases the offspring's chance of surviving . . . at the cost of the parent's ability to invest in other offspring”. He has cleverly used this definition to elucidate many outstanding problems in social ethology^{1–3}. But one of his most appealing usages of it is fallacious.

He states¹: “At any point in time the individual whose cumulative investment is exceeded by his partner's is theoretically tempted to desert”. The context is that of a mated pair of birds, cooperating in the rearing of young. At various times during incubation the female has invested more in the offspring than the male, and the male is said to be tempted to desert. At other times the male's cumulative investment is higher, and the female is then said to be tempted to desert.

If true, this idea would have interesting consequences. A female, in danger of being deserted after copulation, might force a male to invest heavily in her offspring before they are conceived. The price of copulation might be a well built nest and plenty of courtship feeding. Even useless ‘labours of love’ might be imposed as a means of increasing the male's investment before copulation, and therefore of reducing his temptation to desert afterwards. The theory also neatly uses the difference in gamete size to explain why females make better parents than males. As Trivers says¹: “. . . her initial very great investment commits her to additional investment more than the male's initial slight investment commits him”.

The idea has been influential⁴, and it appeals to economic intuition. A government which has invested heavily in, for example, a supersonic airliner, is understandably reluctant to abandon it, even when sober judgment of future prospects suggests that it should do so. Similarly, a popular argument against American withdrawal from the Vietnam war was a retrospective one: “We cannot allow those boys to have died in vain”. Intuition says that previous investment commits one to future investment.

The fallacy can be demonstrated by analogy. Suppose a female has an orphaned baby brother, the same age as her own son. She has only enough food to keep one of the two infants alive. Which should she prefer? Intuition points to the son, but this is not necessarily correct. There are no genetic grounds for preferring either infant: the mother's relatedness⁵ to both is the same, 0.5. A naive application of parental investment theory predicts that the female should prefer the son on the grounds that she has already invested heavily in him: she should not

let all her earlier investment go to waste by preferring the brother, in whom she has invested nothing.

This argument is wrong for it misunderstands why quantity of previous investment is important. A child in whom more has already been invested should be preferred only because he is going to need less investment in the future. This means he can be fledged (or weaned) sooner, and the parent will therefore be free to invest in new children sooner. Our hypothetical female should prefer whichever of the two children has already had most invested in him, regardless of who did the investing, because his future life can be bought more cheaply.

We now return to the original quotation from Trivers. The father and mother have an equal genetic stake in each offspring. Either parent's reluctance to desert at any moment should be related to the quantity of investment still needed to bring the offspring to maturity. This quantity will be negatively related to the amount that has already been invested: it does not matter who did that investing. Trivers almost acknowledges this in another part of the same paper¹, when he suggests that it may pay either partner, even the one who has so far invested most, to desert. This forcible closure of options puts the deserted partner in "a cruel bind". But Trivers's characterisation of the cruel bind is misleading: "... the deserted partner ... has invested so much that he loses considerably if he also deserts the young, even though, which should make no difference to him, the partner would lose even more". The true nature of the cruel bind is this. Regardless of who did the previous investing, the young have had so much poured into them that either partner alone has a good chance of rearing them. To desert after one's partner has deserted is to condemn the young to death; to desert first merely condemns one's partner to make the difficult decision. Genes for deserting first are favoured by natural selection, precisely because genes for deserting second are not. But in either case, the important quantity influencing the decision is quantity of future investment which will be required.

It is of course true that previous investment by a particular parent is relevant to how much he or she can invest in the future: this is the basis of Trivers's definition. But for each particular parent this is a lifetime's calculation: the amount invested in the current offspring is only a small part. There is thus no justification, other than sheer coincidence, for Trivers¹ to draw the cumulative investment curves of his male and female with the same starting baseline. A parent should value the life of a particular child as a function of the proportion that this child represents of his total future reproductive prospects. These future prospects will be negatively correlated with the total amount he has invested in his lifetime, but only negligibly with the amount he has invested in the current particular child.

Does the theory that female coyness might evolve as a means of forcing males to invest early, thereby reducing their temptation to desert later, survive our objection to its parent assumption? This depends on how the majority of females in the population behave. If the majority play the same coy game, desertion tactics will not pay a male: he commits himself to a period of prolonged and costly courtship with a new female before she will let him copulate. If conditions are such that it would pay females to renounce coy tactics, however, the optimal strategy for a male could well be different. This is one of those cases of conflict of interest where the best strategy depends on what others are doing. We therefore suggest that the appropriate conceptual tool is Maynard Smith's⁶⁻⁸ "evolutionarily stable strategy" (ESS). The contest is "asymmetric"⁸, the basis of the asymmetry being sex. We seek a stable combination of male and female strategies, for example, a ratio of 'coy' to 'fast' females which is stable against an equally stable ratio of 'faithful' to 'philanderer' males. Preliminary analysis⁹ suggests that there are conditions in which a stable equilibrium, consisting largely of coy females and faithful males, could evolve. More work is required (J. Maynard Smith, unpublished), but the point we make is that these are the lines along which the thinking must be. We must not simply assume that the partner who has in-

vested least in the current offspring is the one most tempted to desert.

The other suggested consequence of Trivers's theory which we have already mentioned was that the female's high initial investment commits her to continued high investment: thus viviparity, milk-secretion, highly developed maternal care, and so on. Once again, the idea is wrongly expressed. A female is 'committed' to future investment in a child, not because of how much she has already invested in that child, but because of the cost of finishing the task, in relation to how much she would have to invest in order to bring a new child up to the same age.

In any case, the idea of past commitments leading to future commitments had difficulties in explaining the high incidence of paternal care among teleost fish¹⁰. Instances among birds and mammals where the father plays the main parental role are extremely rare; in the teleosts they are commonplace. Why the difference? The following theory makes use of Trivers's "cruel bind" idea, referred to earlier.

Gametes dry up in air, so this has forced terrestrial animals to copulate rather than practise external fertilisation as many fish do. After copulation, the female is left physically in possession of the zygote and while it is still in her body she cannot desert it, but the male can. However fast she lays it, the male is still offered the first opportunity to desert, thereby closing the female's options and forcing her into Trivers's cruel bind. For the reasons already discussed, this tends to lead to the evolution of maternal care.

Fish are not similarly obliged to copulate since there is no danger of their gametes drying up. In such externally fertilising species there is no automatic sense in which the female is left in possession, allowing the male to make his escape. Either sex might thus use the quick-desertion strategy. But there is a possible reason why the male might be more vulnerable to being left 'holding the baby'. Whichever partner spawns first is likely to be one jump ahead, but there is danger in spawning too early; the partner may not be ready. Sperm being lighter than eggs, tend to diffuse faster and so males have more to lose from premature spawning than females. Females can therefore afford to spawn earlier than males, putting them in a strong position to leave the male in possession of the newly conceived zygotes, forcing him into Trivers's cruel bind. This explains the widespread evolution of paternal care in fish. A comparative analysis of the incidence among teleosts¹⁰ of external fertilisation against intromission, in relation to paternal against maternal care, might be rewarding.

In conclusion, a parent 'deciding' whether to desert a child should ask the following questions. How closely related^d is this child to me? How likely is it to survive if I do, and if I do not, desert it? What proportion of my total future reproductive potential does this child represent? How much would it cost me to make a new child equivalent to this one? The parent should ignore its own previous investment in the particular child, except in so far as it affects the answers to these questions. Trivers is largely right, but partly for the wrong reasons.

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Nitrate reductase as a possible predictive test of crop yield

NITROGEN supply is one of the key factors affecting the quality and quantity of crops. The amount of nitrogen fertiliser used is increasing year by year; on a worldwide basis we now use seven times the total fertiliser used in 1939 (ref. 1). In many developed countries the use of excess nitrogenous fertiliser is already causing environmental disturbance, especially in inland rivers and lakes, and represents a potential danger to drinking water supplies. By contrast, in much of the world crops are grown in conditions of nitrogen deficiency. Lacking in each situation is the availability of a reliable method for determining the amount of nitrogenous fertiliser, if any, which should be applied for optimal yield with minimum cost and environmental side effect, or for maximum use of limited resources. We have found that early season assays of nitrate reductase provide such a method.

Usually, whatever the source of nitrogen fertiliser, soil conditions ensure that nitrogen enters the plant predominantly as nitrate. The first and rate limiting step in nitrate utilisation is catalysed by the inducible enzyme, nitrate reductase² and Hageman's group in Illinois have demonstrated a correlation between nitrogen supply, nitrate reductase activity integrated throughout the year and grain yield in wheat³ and maize⁴. In these experiments, which were conducted in suboptimal nitrogen conditions, addition of further nitrogen, especially during the early part of the growing season, led to increases in nitrate reductase activity and in final grain yield.

We have examined the possibility of using nitrate reductase assays, early in the development of the crop, as a predictor of ultimate grain yield. In the first series of experiments wheat plants (Hope/Chinese Spring substitution line 7B) were grown in sand culture in controlled conditions in growth rooms, at a number of defined nitrate levels. Nitrate reductase activity in the uppermost fully expanded leaf (which was found to have the highest nitrate reductase activity (R. Hallam and G. C. Blackwood, unpublished results)) was measured weekly *in vitro* and the effect of nitrate concentration on nitrate reductase activity was compared with the effect on the final yield. The data are shown in Fig. 1. There is a good correlation between nitrate reductase and yield ($r=+0.90$, $n=7$) at the earliest date of measurement, except at high (50 mM) nitrate concentrations. Similarly, there was a good correlation between grain yield and grain protein ($r=+0.969$, $n=8$) indicating that increasing nitrogen supply had no effect on the protein content of the grain. Furthermore, there were no significant changes in amino acid composition. Thus early nitrate reductase activity provides a good estimate of ultimate grain yield.

The critical test of a predictive assay is its successful use on crops growing in the field. To be sure of nitrogen deficient conditions for such a test, experiments were carried out (in collaboration with Dr. A. E. M. Hood of ICI's Jealott's Hill Research Station) on long standing nitrogen deficient plots at West Isley, Berkshire. Plots were treated with 0, 75, 112 or 150 kg ha⁻¹ of nitrogen and measurements of nitrate reductase made early, in the middle and late in the growing season. The crop was spring barley var. Julia. As before, samples for assay consisted of the uppermost fully expanded leaves and these were collected in the morning and stored at ambient temperature

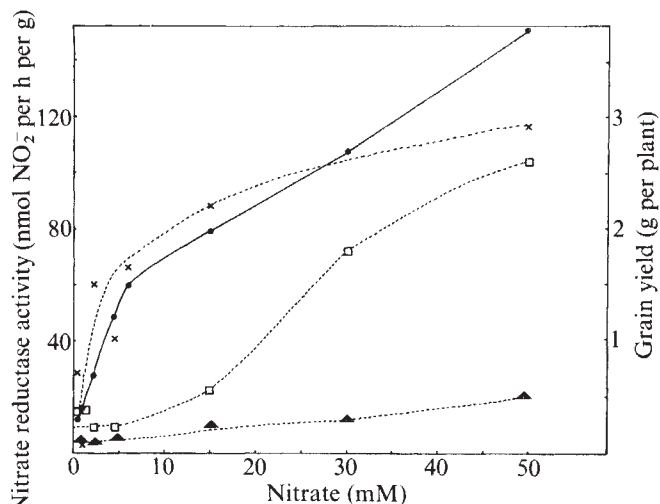


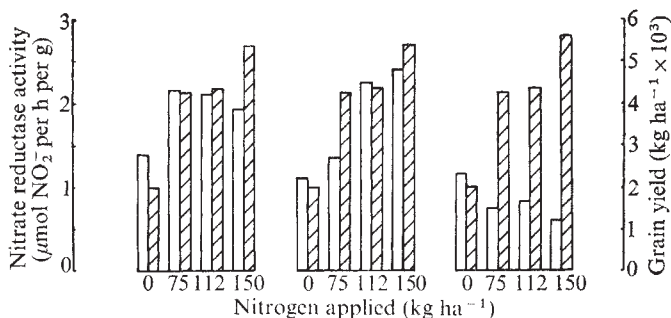
Fig. 1 Nitrate reductase activity as related to nitrate concentration in wheat (*Triticum aestivum* Hope/Chinese Spring, substitution line 7B). The enzyme was extracted from the first fully expanded leaf and assayed according to Filner⁷ (x, 2 weeks; □, 3 weeks; ▲, 4 weeks after sowing). For comparison the final grain weight per plant is shown (●). Plants were grown in sand culture on Long Ashton growth medium⁸ adjusted to give the desired NO₃⁻ concentration while maintaining constant ionic strength. Day length was 16 h; temperature 25/17 °C and light intensity 35 W m⁻².

for several hours before nitrate reductase was determined by an *in vivo* assay in the laboratory, later in the day. Storage of intact leaves in this way did not significantly alter the enzyme activity. This is important if the test is to be of any practical use.

The activities of nitrate reductase at the three times of measurements are compared with the final yield in Fig. 2. The data show that, on the earliest date of estimation (3–4 leaf stage), less than maximal nitrate reductase activity is a reliable predictor of suboptimal yield. This is in agreement with the data obtained in the growth room. Amino acid composition was unaffected by the treatments. Once again, there is an increase in yield obtainable at nitrogen levels in excess of those required for maximal nitrate reductase induction. As with the growth room experiments, however, the increase in yield at these high nitrogen levels is far less in relation to added nitrogen than that obtained at lower levels where yield is correlated with nitrate reductase activity.

Although the measurements reported here were carried out in the laboratory, the *in vivo* assay can easily be used in the field. An alternative method which we also investigated,

Fig. 2 Nitrate reductase assayed on three dates (open columns) and final grain yield (hatched) in barley (*Hordeum vulgare*, var. Julia) plants. Enzyme activity was assayed *in vivo* by the method of Stewart *et al.*⁹. Standard errors: enzyme activity, $\pm 0.155 \mu\text{mol NO}_3^-$ reduced per h per g; yield, $\pm 238 \text{ kg ha}^{-1}$.



measurement of tissue nitrate, is far more complex and less reliable, and although it yielded similar results in our conditions, it is known that NO_3^- accumulation without related nitrate reduction can occur^{5,6}. Although the nitrate reductase measurements do not correlate perfectly with final yield they provide a useful test for future yield except at the highest nitrogen levels and there is at present no remotely reliable alternative. Furthermore preliminary results of Hallam and Blackwood (unpublished) suggest that by careful selection of tissue for assay a correlation which is less time dependent can be obtained. The increasing cost and scarcity of natural resources, the important need to conserve our environment and a continuously increasing worldwide requirement for protein may well make such a test less than a luxury in the near future.

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Possible origin for insoluble organic (kerogen) debris in sediments from insoluble cell-wall materials of algae and bacteria

KEROGEN is an inert amorphous organic material insoluble in organic solvents, mineral acids, and bases¹. In spite of its abundance, the precise molecular structure of this insoluble organic material has long remained an enigma. Structural determinations are complicated by the fact that the structure of any kerogen from different samples varies depending on the types of organic material originally responsible for its formation, and variations in the environmental conditions experienced during and after its formation. We have shown that kerogen-like material can be isolated from recently deposited algal mats. On oxidative degradation this material gives rise to products similar in nature to those obtained by degradation of ancient kerogens known to be of algal origin^{2,3}. These results imply that kerogen-like precursors or proto-kerogens, are formed very rapidly on a geological time scale and are found in certain types of recent sediments rich in organic matter.

We have now carried out similar degradation studies on some pure cultures of blue-green and green algae and bacteria, to determine whether the types of degradation products produced would be comparable to those previously obtained from the degradation of ancient kerogens known to be of an algal origin. Microscopic examination of ancient shales, such as the Green River oil shale⁴ or Tasmanian tasmanite⁵, clearly shows that the bulk of the organic material in these shales is derived from blue-green or green algae, bacteria, fungi and other microorganisms. It is the organic material from the residues of these organisms which represents the starting point in the formation of algal kerogens, although major changes can occur in the structure of the residues after deposition in the sediments and the formation of kerogens. A study of the insoluble organic material from contemporary blue-green and green algae, and

bacteria, however, should provide valuable comparative information on the origin and possible method of formation of algal kerogens.

The inertness of kerogen is testified to by the fact that it is the residue which remains after acid treatment of a rock or shale sample to remove minerals, such as carbonates and

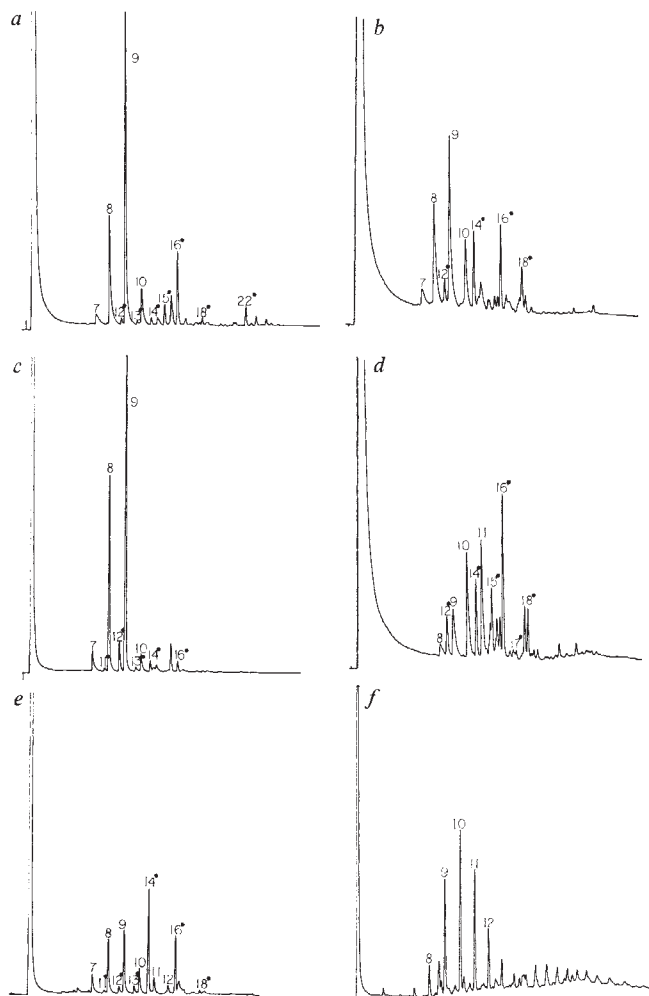


Fig. 1 Gas chromatograms of the unbranched oxidation products obtained from the oxidative degradation of the insoluble residues from the algae and bacteria as indicated. The Tasmanian tasmanite kerogen fraction contains the ether soluble oxidation products from a 60-h oxidation of the kerogen. Numbers indicate that these peaks have been identified as α,ω -dicarboxylic acids with the corresponding number of carbon atoms. Peaks labelled 14* and so on indicate that these peaks have been identified as normal acids with the corresponding number of carbon atoms. Note that in all the chromatograms, except those from the *Anacystis* and *Rhodospirillum rubrum*, the distributions maximise at the C_9 - α,ω -dicarboxylic acid. In the chromatogram from the Tasmanian tasmanite, the products maximise at the C_{10} - α,ω -dicarboxylic acid. Note that the Tasmanian tasmanite chromatogram represents only the ether soluble degradation products; the normal acids, normally extracted with heptane, are not present in this chromatogram. Note, however, that the major degradation products from these organisms are basically the same as those from certain ancient kerogens although there are minor differences in the distributions. These chromatograms were obtained using a Varian 2700 gas chromatograph equipped with a FID detector. A 15-foot $\times$ 1/16-inch stainless steel column packed with Dexsil 300 on Gas Chrom Q was used for analysis in the following conditions: oven temperature 100-280 °C, 6° min⁻¹; injector temperature 280 °C; detector temperature 250 °C, and a helium flow rate of 15 ml min⁻¹. Computerised gas chromatography-mass spectroscopy analyses were carried out on a DuPont 491-2 mass spectrometer coupled with a Varian 204 GC. Mass spectral data were acquired using a DuPont 21-904 data system. *a*, *Chlorococcoides*; *b*, *Chlorobium*; *c*, *Phormidium*; *d*, *Rhodospirillum rubrum*; *e*, *Anacystis*; *f*, Tasmanian tasmanite kerogen.

Table 1 Elemental analyses, yields and ratios of branched-unbranched products obtained from the insoluble organic residues of the organisms indicated

Organisms	Elemental analyses of residues						A*	B†	Ratio of branched-unbranched products
	%C	%H	%N	%O	%S	%Ash			
Algae									
<i>Nostoc muscorum</i> (blue-green)	45.4	6.1	14.0	28.6	0.7	5.2	2	16	13:1
<i>Anacystis nidulans</i> (blue-green)	48.9	7.1	13.2	29.6	0.5	0.7	2	45	5:1
<i>Phormidium luridum</i> (blue-green)	49.7	6.7	13.0	29.0	0.7	0.9	2	8	4:1
<i>Chlorella pyrenoidosa</i> (green)	51.4	6.8	13.2	27.7	0.7	0.2	2	7	5:2
Bacteria									
<i>Rhodospseudomonas spheroides</i> (photosynthetic)	52.5	6.9	11.3	28.4	0.8	0.1	1	6	2:1
<i>Chlorobium</i> (sulphur bacteria)	50.5	6.9	11.0	30.7	0.6	0.3	1	6	4:1

*Amount (g) of insoluble residues used in the oxidation reaction.

†Yields (mg) of products obtained on extraction of the oxidation reaction mixture with heptane and ethyl acetate.

silicates, and exhaustive extraction of the remaining residue with organic solvents to remove any soluble lipid material¹. Any humic material can also be removed from this residue by treatment with potassium hydroxide. Kerogen is the most abundant reservoir of organic carbon on the Earth, and as an example of its abundance it has been estimated that there are 3×10^{15} tons of kerogen, compared with 5×10^{12} tons of coal, in the Earth's crust⁶.

Tissot *et al.* have characterised kerogens as originating either from algal debris, humic or plant debris, or from a mixture of both types of material. Of the two extremes, algal kerogens are generally considered to have an aliphatic-type structure and humic kerogens an aromatic structure⁷. Many chemical and physical degradation studies carried out on kerogens from ancient shales and sediments have yielded valuable, although limited, structural information. The reagents used in chemical degradation studies have included oxidative reagents such as potassium permanganate⁸, chromic acid⁹, and ozone¹⁰, and reductive reagents such as lithium aluminium hydride¹¹. Pyrolysis is another technique which has been used to degrade kerogens¹². The majority of this work has been carried out on kerogens isolated from such ancient shales as the Green River oil shale⁹ (Eocene, 50×10^6 yr) or Tasmanian tasmanite⁵ (Permian, $220\text{--}275 \times 10^6$ yr), (see ref. 8 for additional examples of kerogens examined).

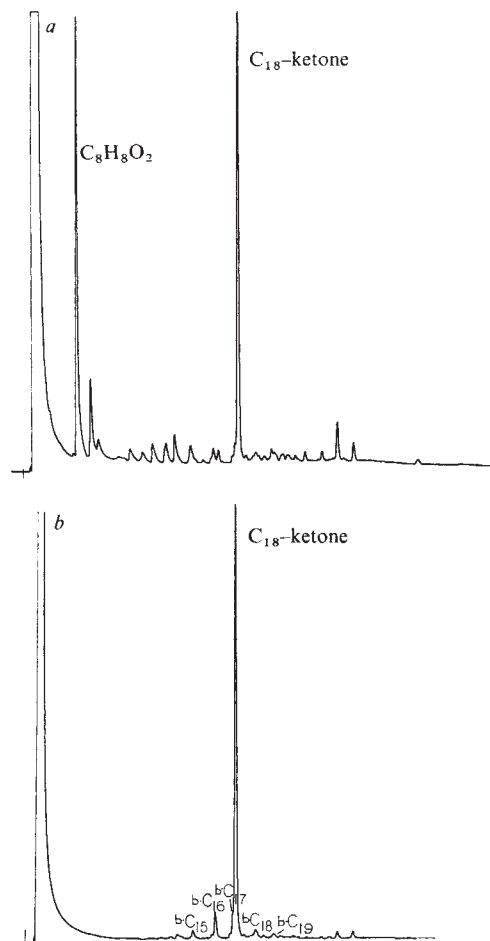
We have carried out degradation studies on the insoluble organic residues obtained from cultures of the algae and bacteria shown in Table 1. The conditions in which these cultures were prepared and the results of the soluble lipid analyses have been described previously^{13,14}. Freeze-dried samples of algae and bacteria (Table 1) were treated as for the isolation of kerogen from an oil-shale sample. Each sample was exhaustively extracted with a mixture of toluene-methanol (1:1, using ultrasonication) and after 16 successive extractions the organic extracts were colourless, indicating removal of all readily soluble pigments and lipid material. (It was assumed that ultrasonication was sufficient to rupture the cells; however, the residues were not examined under the microscope either at this stage or after the HCl treatment. The possibility of some intracellular lipids remaining after extraction and hydrolysis cannot be completely excluded.) The residues were subsequently hydrolysed with 6 N HCl and, after centrifugation and removal of the aqueous layer, exhaustively extracted again with organic solvent. In the case of an oil shale, the kerogen is further concentrated by treatment with 48% HF and HNO₃ to remove silicates and pyrites, respectively¹. This was unnecessary, however, in the case of these pure cultures for obvious reasons.

The resulting residues, after drying, were subjected to alkaline permanganate oxidation described previously¹⁵. The organic oxidation products were extracted from the reaction mixture with heptane and then ethyl acetate, and these extracts subsequently combined. After methylation of the extracts by BF₃/MeOH, they were fractionated into branched and unbranched fractions by urea adduction. These fractions were subsequently analysed by gas chromatography, and com-

puterised-gas chromatography-mass spectrometry. Components in the various fractions were identified from their relative retention times, coinjection with standards where possible, interpretation of individual mass spectra, and comparison of mass spectra with authentic spectra wherever possible.

Table 1 shows the elemental analyses of the organic residues from the cultures, the yields and relative amounts of un-

Fig. 2 Gas chromatograms obtained of the branched oxidation products from *Phormidium luridum* (a) and *Chlorella pyrenoidosa* (b). Conditions as in Fig. 1. These two chromatograms illustrate the major features seen in the branched component fractions from all the samples. The C₁₈-ketone was the dominant product in all cases and the second major product was benzoic acid (as the methyl ester derivative used for gas chromatographic analysis). *Chlorella pyrenoidosa* was the exception, in that it was the only sample on degradation to give the isoprenoid acids (the label b-C₁₆ indicates the peak corresponding to the C₁₆-isoprenoid acid) shown in this chromatogram.



branched and branched components after oxidation and fractionation. The yields of oxidation products are noticeably low. This is not surprising and was indeed anticipated since it is known that the cell-wall material of blue-green algae consists mainly of protein and polysaccharides¹⁶. On oxidation this will give rise to water-soluble amino acids and carbohydrates which are removed in the aqueous layer during the extraction of oxidation products. These products were not examined.

Figures 1 and 2 show the chromatograms obtained from the analyses of the oxidation products of the insoluble residues and indicate the components which have been identified. The unbranched fractions were dominated by α,ω -dicarboxylic acids in the range C_6 – C_{12} with maximum at C_9 and n -acids in the range C_{11} – C_{18} with a maximum at either C_{14} , C_{16} or C_{18} (Fig. 1). These acids were not found in the soluble lipid extracts (except for certain of the n -acids^{13,14}) of the algae or bacteria and therefore do indeed represent degradation products of this insoluble cell debris. The branched fractions were less complex than the above mentioned fractions and in the majority of cases consisted of benzoic acid, a C_{18} -isoprenoidal ketone (6,10,14-trimethylpentadecan-2-one), and in one example, *Chlorella pyrenoidosa*, of C_{15} , C_{16} , C_{17} , C_{18} and C_{19} isoprenoidal acids in relatively small amounts. These degradation products in turn bear a remarkable resemblance to those obtained from the oxidative degradation of ancient kerogens known to be of algal origin—for example, kerogen from the Green River oil shale⁸ or Tasmanian tasmanite⁵. The individual mixtures of products in this study are not anywhere near as complex as those from ancient kerogens. This is not surprising, however, when one realises the differences in age, thermal history, and environmental conditions that have been experienced by the ancient kerogens at the other end of the geological time scale.

There are several possible origins for these degradation products. First, they could be derived from intracellular chlorophyll, which is not removed during the extraction process. This would tend to support the theory of Oehler *et al.*¹⁷ that intracellular chlorophyll becomes grafted on to cellular macromolecules, and with increasing maturation finally gives rise to kerogen. It would also support the theory of Burlingame and Simoneit¹⁸, who proposed that the presence of the C_{18} -isoprenoid ketone in the degradation products of Green River shale kerogen indicated cross linking at the allyl rearrangement centre of phytol (derived from the phytol side-chain of chlorophyll) during its incorporation into the kerogen moiety.

Second, it is possible that the degradation products are derived from cellular macromolecules which on oxidation give rise to the above mentioned products plus other water-soluble products not examined here. This would support the theory that it is this residual polymeric debris which is the source of the major building blocks of kerogen. With increasing time additional soluble lipid material can become condensed to this basic framework by irreversible chemical reactions, possibly catalysed by clay minerals in the local sedimentary environment. This mechanism plus condensation between the polymeric debris itself would give rise to the kerogen matrix as found in many sedimentary environments.

To extend our theory that the insoluble residues from algae and bacteria are the basic building blocks for kerogen, maturation experiments attempting to incorporate ¹⁴C-labelled biolipids into these residues are underway. It is anticipated that in simulated geological conditions, the degree of cross linking within the insoluble residues will increase and incorporation of the ¹⁴C-biolipids will also occur.

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Prepupal larval mosaics in *Drosophila melanogaster*

THE metamorphosis of insects provides a model system for the study of hormone action. In *Drosophila*, metamorphosis is initiated by the formation of a puparium with a rigid cuticle that becomes tanned early in the prepupal period. Subsequently many larval tissues degenerate and the imaginal tissues develop to produce the adult insect. These processes are initiated in *Drosophila* by the steroid moulting hormone, β -ecdysone¹. We are interested in the mechanisms by which β -ecdysone elicits adult development. The recovery of mutants which cannot respond to the hormone would facilitate investigation into the nature of the action of β -ecdysone. We have isolated systematically a series of late larval and prepupal X-linked lethals (ref. 2 and unpublished results of I.K. *et al.*), about 15% of which are non-pupariating (*npr*) lethals in which metamorphosis is not initiated. We have investigated whether the *npr* condition is a result of the autonomous inability of the mutant target tissues to respond normally to β -ecdysone or rather is a stage-specific failure in the production of the hormone. Our results indicate that in most *npr* lethals the tissue cannot respond to the hormone. Furthermore, we estimate that there are 100–200 such lethals in the genome of *D. melanogaster*.

One way to investigate whether there is a failure in response to β -ecdysone or a loss of its synthesis for X-linked recessive lethals is to produce gynanders containing patches of male (X/O) tissue in which the recessive lethal is hemizygous (hence potentially expressed) and female (X/X) tissue in which the recessive gene is heterozygous (hence, phenotypically unexpressed). Production of such gynanders in *D. melanogaster* is accomplished routinely by crossing ring-X males with females heterozygous for the lethal. In the progeny two types of females are produced; one type carrying the ring X and a lethal-bearing X chromosome, and a second type carrying the ring X and a non-lethal-bearing X (usually a balancer chromosome). During early nuclear divisions in the embryo the ring X chromosome is occasionally lost from some cells with the resultant production of an organism containing both male (X/O) and female (X/X) tissue. For progeny carrying the lethal one would predict that if the *npr* condition were autonomous there

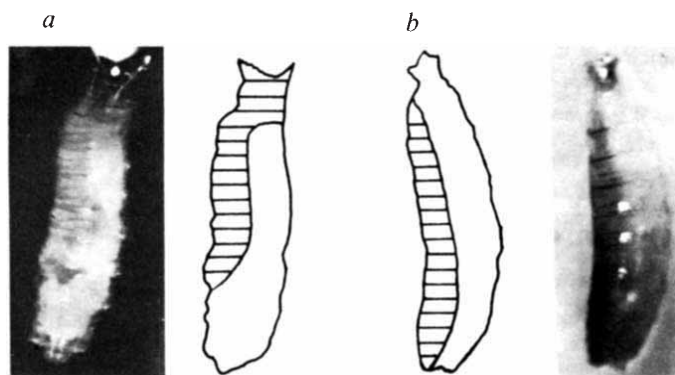


Fig. 1 Photographs and schematic diagrams of larval-prepupal mosaics; *l(1)d. norm.-1^a/ring (1)2* (b) and *l(1); d. norm.-12/ring 1(2)* (a). See ref. 3 and the legend to Fig. 2 for a description of genetic nomenclature and stocks. In the schematic diagrams the cross hatched regions represent tanned puparial tissue.

should be no adult gynanders produced, but instead developmentally arrested mosaics composed of prepupal (X/X) and larval (X/O) tissue. The latter condition would be identifiable readily by the presence of tanned puparial tissue sharply separated by distinct boundaries from white larval tissue.

Such larval-prepupal mosaics were obtained in all of six *npr* mutants investigated. Examples are shown (Fig. 1) involving two independently recovered *npr* lethals. There were sharp boundaries between the larval and the pupariated tissue. For three of the six mutants investigated there was good evidence that some of the mosaics resulted from gynander production. In one case, involving *l(1)d. norm.-12*, the maroon-like marker had been added by recombination to the chromosome carrying the lethal. Since *ma-1* lacks aldehyde oxidase activity, male (X/O) tissue will be deficient in the enzyme activity and hemizygous for the lethal, but female (X/X) tissue will have aldehyde oxidase activity and be heterozygous for the lethal. Histochemical identification of the tissue distribution of the enzyme activity using Janning's method⁴ revealed perfect correspondence between aldehyde oxidase negative and non-pupariating tissue as expected for male (X/O) tissue in a gynander. (Details of experiments using *ma-1* will be published elsewhere.) In a mosaic involving *l(1)d. norm.-1^a*, the anterior of the mosaic was larval, had yellow mouth hooks and thus could be identified as male (X/O) tissue because of the presence of the recessive *yellow* marker on the lethal-bearing X chromosome. The posterior, which had pupariated, contained ovaries, and thus was composed of female (X/X) tissue. In another case, involving *l(1) discless-1*, the larval region

of the mosaic contained no imaginal disks while the pupariated region had normal imaginal disks. As a control, females carrying the ring X and a recessive X-linked lethal which causes death after puparium formation, *l(1)d. norm.-6*, failed to produce any larval-prepupal mosaics in contrast to the *npr* lethal *l(1)d. norm.-1^a* (Table 1). Adult gynanders of female sibs carrying a non-lethal X chromosome were found easily in both cases. Adult gynanders have not been found for any of the six *npr* mutants investigated. Nevertheless, because of the absence of appropriate markers, we lack evidence that some larval-prepupal mosaics are the result of gynander production for three of the six mutants investigated. Caution in interpretation is necessary because larval-prepupal intermediates can be produced by mistreating larvae (for example, by prolonged immersion in water). The results indicate, however, that 50% or more of X chromosome *npr* mutants involve autonomous defects in the target tissue.

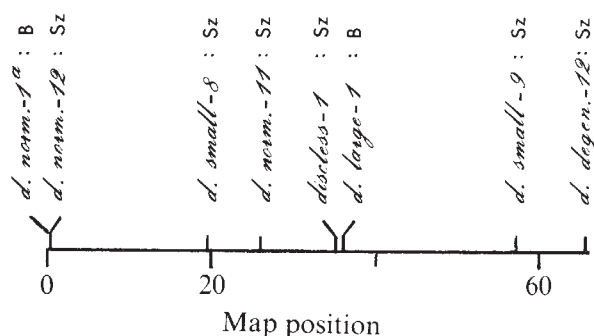


Fig. 2 Map positions of X chromosome *npr* mutants. The methods used for determining map positions are presented elsewhere (ref. 2 and unpublished results of I.K. *et al.*). Mutants isolated in Szeged are indicated by Sz; those isolated in Berkeley by B. The lethals are phenotypically described in terms of the condition of the imaginal disks. Mutant nomenclature: *l(1)*: lethal, first chromosome, has been omitted; *d.*: disk; *norm*: normal; *degen.*: degenerate.

Eight *npr* mutants have been mapped and assigned approximate map positions (Fig. 2). There are at least six (probably seven) loci involved. Based on map position and phenotypic similarity we conclude that two mutants, *l(1)d. norm.-12* and *l(1)d. norm.-1^a* are allelic and located slightly to the right of *yellow* (ref. 2 and unpublished results of I.K. *et al.*). Although *l(1) discless-1* and *l(1)d. large-1* map close to each other they are phenotypically very different and probably not allelic. From the above information, assuming that the induction of mutants follows a Poisson distribution, it is possible⁵ to estimate the total number of *npr* genes. We calculate that the X chromosome contains about 20 *npr* genes and the whole genome of *D. melano-*

Table 1 Gynander recovery using two late-acting lethals

Cross	Males		Offspring				Larval prepupal mosaics
	Adult <i>Bin sn</i>	Adult lethal	Adult non-gynanders	Adult gynanders	Adult non-gynanders	Adult gynanders	
<i>npr</i> -lethal; <i>ring (1)2/Yy⁺♂♂</i> × <i>l(1)d. norm.-1^a/Binsn♀♀</i>	458	0	16	25	9	0	12
	% gynanders:		58% (25/41)		57% (12/21)		
Prepupal-lethal; <i>ing (1)2/Yy⁺♂♂</i> × <i>l(1)d. norm.-6/Binsn♀♀</i>	503	0	22	20	19	1	0
	% gynanders:		47% (20/42)		5% (1/20)		

For a description of genetic nomenclature and stocks see ref. 3 and the legend to Fig. 2. About 10 aged, virgin females were mated in vials with five males. Flies were transferred to fresh vials every 2 d for 2 weeks. Vials were examined twice daily from the emergence of the first offspring until no progeny or live larvae were found over 48 h. Stocks were maintained on standard *Drosophila* medium at 25°C.

gaster about 100. A similar estimate can be obtained from the percentage of late acting lethals that are *npr* mutants. Of the late acting lethals on the X chromosome 17% (9/53) have the *npr* phenotype (ref. 2 and unpublished results of I.K. *et al.*). Shearn⁵ estimates from studies on third chromosome lethals that there are about 900 genes in the *Drosophila* genome which cause late lethality. From this number we estimate that there are about 150 *npr* genes (17% of 900). Kiss and coworkers (unpublished) based on results involving late acting X chromosome lethals, estimate that there are about 750 late acting lethals in the *D. melanogaster* genome, about 125 of which will be *npr* lethals. Although these estimates are approximations, they all suggest that the total number of *npr* genes is small, that is 100–150. Thus, it should be possible to recover mutants of all such X-linked genes and probably of all those on the autosomes as well.

In cases where larval and prepupal mosaicism occurs, the presence of the two types of tissue together in the gynander indicates that the β -ecdysone titre has reached sufficient levels to initiate metamorphosis. Thus, in these cases the mutant tissue cannot respond normally to the hormonal stimulus. We estimate the total number of *npr* genes preventing a specific response of a target tissue to the hormonal stimulus to be 75 or more. But the bases of action of the mutants may be separable into at least three different classes, and all may not be involved in the response to β -ecdysone *per se*. In addition to the category of mutants in which functions necessary to produce a puparium are lost, one can imagine a category of mutants affecting the competence of the epidermal tissue to become programmed to respond to the hormone by puparium formation and a third category in which premature degeneration of the tissue precludes puparium formation. As *in vitro* test systems are available, involving larval target tissues (salivary glands⁶, imaginal disks⁷ and larval cuticle⁸), it should be possible to investigate the effects of the autonomous *npr* mutants at the cellular and molecular level and distinguish between the above possibilities.

The production of gynanders with both larval and prepupal tissues has implications for studies involving the recovery of adult gynanders. The inability to respond to β -ecdysone implicit in the autonomous *npr* condition is apparently not limited to the larval epidermis, but involves other larval tissues². The maintenance of a substantial fraction of tissue in the juvenile form while another fraction proceeds into and through metamorphosis in all likelihood will result in the failure of eclosion of the adult fly. Thus, even if the effects of the lethal gene are limited to larval tissues, the recovery of adult gynanders might be rendered highly unlikely. Such a case is provided by *l(1) d. norm.-1^a* (Table 1) and also by *l(1) d. norm.-12* in which no gynander tissue has been found in carrier adult females (ring/lethal-bearing X) but in which mutant disks develop (albeit poorly) along with the host after transplantation into the haemocoel of a wild type larva².

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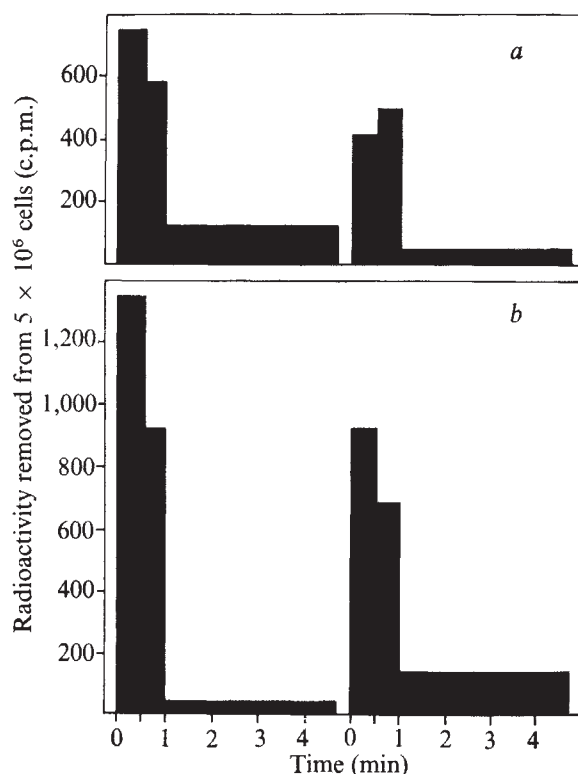
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Determination of cell shape by a cell surface protein component

CONSIDERABLE evidence derived from the study of animal cells cultured *in vitro* implicates the cell surface in the regulation of cell division and in the expression of the phenotypic state of the cell. Cell surface is a broad term used to indicate the lipid bilayer of the cytoplasmic membrane containing intrinsic (stably integrated) and peripheral (easily removable) protein constituents plus carbohydrate complexes^{1–3}. There are indications that some of the intrinsic constituents are of fundamental importance in certain physiological processes such as ion transport and transport of metabolic precursors^{4–8}. On the other hand, it is not known whether components that are more loosely bound to the cell surface play any specific role in cell physiology because no cellular function has so far been examined in relation to such components. We report here that determination of cell shape is affected by a cell surface protein component easily detachable from the cell exterior.

Secondary or tertiary mouse embryo fibroblasts (MEF) derived from the C57BL/6 strain were grown in Eagle's BHK medium supplemented with 5% foetal calf serum. A medium of the same composition and osmolarity as that of the culture medium, but containing no serum (serum-free medium, SFM), was used for washing and incubation during the experiments. Our first aim was to investigate whether any material could be removed from the cell surface without the aid of proteolytic enzymes or chelating agents. Cultures that had been washed

Fig. 1 *a*, Cells labelled with ¹⁴C-amino acid mixture. *b*, Cells labelled with ³H-D-glucosamine. Histograms on the left represent radioactivity associated with non-dialysable material from cultures 20 h after seeding; histograms on the right from cultures 72 h after seeding.



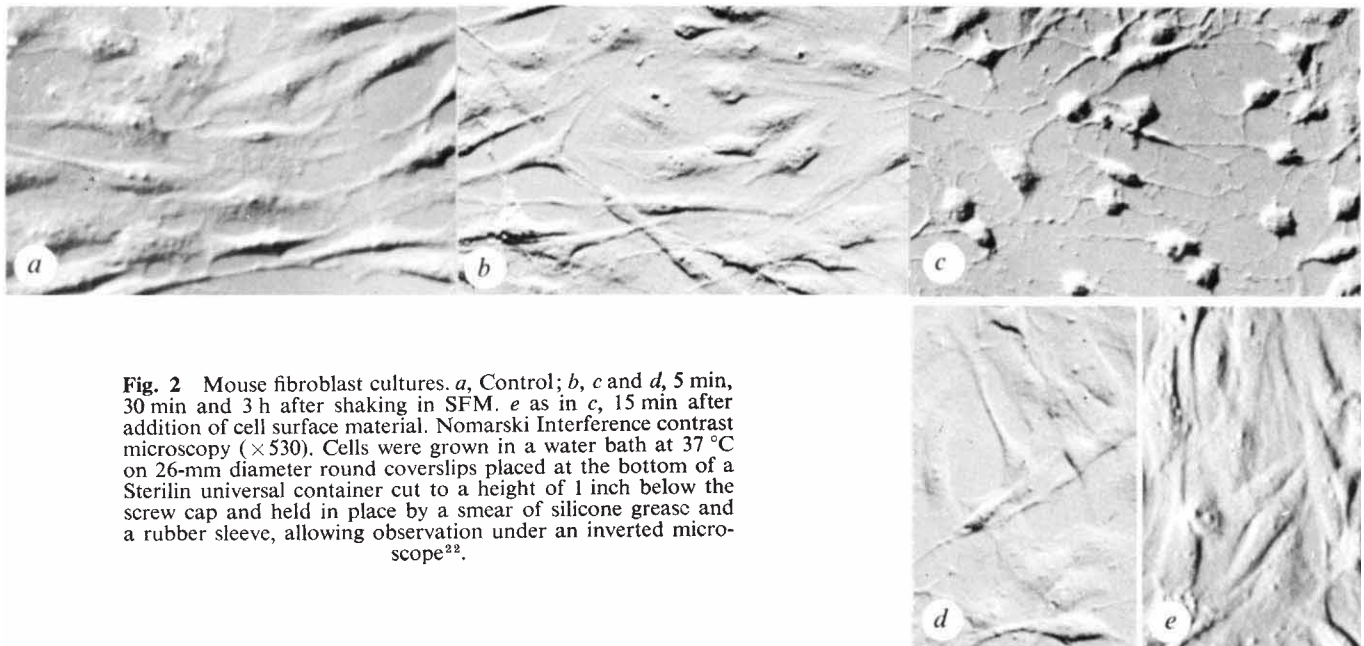


Fig. 2 Mouse fibroblast cultures. *a*, Control; *b*, *c* and *d*, 5 min, 30 min and 3 h after shaking in SFM. *e* as in *c*, 15 min after addition of cell surface material. Nomarski Interference contrast microscopy ($\times 530$). Cells were grown in a water bath at 37°C on 26-mm diameter round coverslips placed at the bottom of a Sterilin universal container cut to a height of 1 inch below the screw cap and held in place by a smear of silicone grease and a rubber sleeve, allowing observation under an inverted microscope²².

four times were incubated in SFM containing either ^{14}C -protein hydrolysate (50 mCi per matom C, $1\ \mu\text{Ci ml}^{-1}$) or ^3H -D-glucosamine-HCl ($3\ \text{Ci mmol}^{-1}$, $2.5\ \mu\text{Ci ml}^{-1}$). After about 20 h the radioactive medium was removed, the cells were washed and fresh SFM, a quarter the original volume, was added. The cultures were then placed on a flat surface and submitted to horizontal shaking at 20°C using a frequency of three excursions per second and an amplitude of 5 inches. The medium from each culture was collected at 30-s intervals, centrifuged at $2,000g$ for 10 min, dialysed in SFM and assessed for radioactivity. Figure 1 shows that both protein and carbohydrate containing macromolecules were freed easily, and that most of the radioactivity was removed with the pattern of a distinct entity within the first minute. A 3-h ^{51}Cr release assay⁹ showed no difference between shaken and unshaken cultures, indicating that cell integrity had not been affected by the mechanical perturbation. In fact, the ease with which cellular material could be removed was demonstrated by gentler procedures. Simple SFM washings yielded variable but sizeable amounts of labelled components.

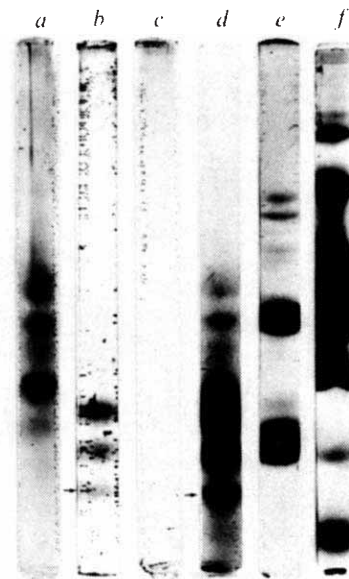
The most obvious and immediate parameter investigated in studies of cell behaviour is form. When returned to an incubator at 37°C , cells that had been washed or shaken showed a degree of transient rounding. This could be reproduced with uniformity of response and with a consistent kinetic pattern if synchronised tertiary S phase fibroblasts were used and if the shaking procedure described above lasted for a standard period of 1 min. A reduction of the cell projected area and an increase of thickness was noticeable soon after incubation, and was followed by a progressively increasing rounding. At 20 min cells were markedly round with elongated cytoplasmic processes extending in thin ramifications. The rounded state was maintained for a short time, then, gradually, within about 3 h, the cells reverted to their original appearance. Figure 2 *a-d* shows some of the stages in the described sequence of events. Rounding did not occur nor could reflattening take place if the cells were left at or transferred to room temperature.

Among the factors that contribute to determination and maintenance of form are the architectural structures of the cell surface. It seems unlikely, however, when the phenomenon we describe is seen in the light of a cause-effect relationship, that constituents that are stably integrated in the surface membrane could have a primary (first in time) role in the initiation and modulation of the morphological changes as the principle of variation is implicit to the concept of modulation. Thus the induction of rounding that follows shaking in SFM is more likely to be a consequence of the removal of extrinsic factors

required for the maintenance of cell shape and reversion to the flattened state to be due to the cell's ability to replace the removed components. To test this possibility we investigated whether the shaken material had any effect on cell morphology. The fraction removed from the cells after 1 min of shaking (Fig. 1) was concentrated by ultrafiltration through Amicon PM-10 membranes (10,000 molecular weight cutoff) to a protein content of $0.5\ \text{mg ml}^{-1}$. Dilutions of this preparation in SFM down to about $50\ \mu\text{g}$ protein per ml prevented the induction of cell rounding when added immediately after shaking and caused rapid reflattening when added to rounded cells (Fig. 2*e*).

We used chromatographic separation to investigate whether this effect was due to the macromolecular complex as a whole or to any components in particular. Concentrated material (2 mg) was loaded on a column of Sephadex G-75 pre-equilibrated and eluted with SFM at 4°C . Examination of the fractions

Fig. 3 Coomassie blue staining of 5% SDS-polyacrylamide gel electropherograms. *b*, Active fraction from Sephadex column; *a* and *c*, neighbouring fractions; *d*, unfractionated shaken material; *e*, immunoglobulin heavy and light chain (25,000 molecular weight bottom band); *f*, foetal calf serum. Arrow indicates band unique to active preparations. *a* to *c*, high contrast photography.



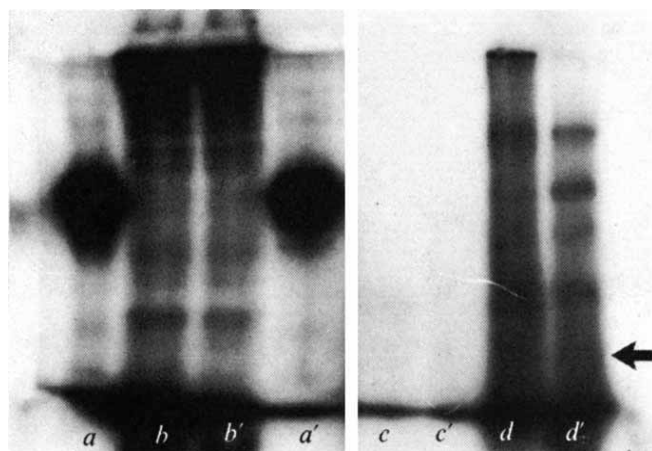


Fig. 4 Autoradiographs from 7.5% SDS polyacrylamide gel electrophoresis of iodinated preparations run in duplicate. *c* and *c'*, Active fraction from Sephadex column; *b* and *b'*, whole cell; *d* and *d'*, unfractionated shaken material; *a* and *a'*, foetal calf serum. Arrow indicates position of migration of corresponding band in the various preparations. No corresponding band was present in the serum.

showed that only one (no. 13; 20–25,000 expected molecular weight) had the protective activity detectable in the original material. The protein content of this fraction was about $10 \mu\text{g ml}^{-1}$ but a 1:2 dilution still retained activity. This fraction (no. 13) and the two neighbouring fractions (nos 12 and 14) were concentrated and examined by sodium dodecyl sulphate–polyacrylamide gel electrophoresis¹⁰ in parallel with the original unfractionated material and with a sample of the foetal calf serum used for cultivating the cells. Comparison of the pattern of migration of the banded components after staining of proteins showed that only one band (<25,000 molecular weight) was unique to those preparations able to maintain or reinduce the flattened state (Fig. 3). The cellular origin of this component was endorsed by the absence of a corresponding band in the serum. Staining of the gels by the periodic acid–Schiff method for carbohydrate¹¹ did not permit detection of the active component, indicating the possible absence of sugar residues in the protein molecule.

Although the procedure used clearly indicates that several cellular components were loosely attached to the cell exterior and could easily be removed, final identification of the active factor on the cell surface was sought by lactoperoxidase-catalysed iodination (^{125}I) of the intact cells, a technique used to label selectively membrane proteins exposed at the cell surface^{12,13}. Parallel ^{125}I labelling of unfractionated material, of Sephadex fractions and of serum proteins was carried out by the

chloramine-T method¹⁴. Lactoperoxidase iodination was done in monolayer cultures that had been washed three times in phosphate-buffered saline (PBS, containing Ca^{2+} and Mg^{2+}) to remove serum. After iodination the cells were washed twice in PBI (sodium iodide instead of chloride as in PBS) containing a protease inhibitor and then collected by scraping. Comparison of the autoradiographs after polyacrylamide slab gel electrophoresis showed a corresponding band in the active Sephadex fraction, the whole cell preparation and the concentrated material removed by shaking in SFM (Fig. 4). No correspondence was found with protein constituents of the iodinated serum. The presence of the common band and its relevance in relation to the effect exerted on morphology was corroborated by the results obtained from densitometric analysis (courtesy of Dr P. Gray, Imperial College, London) of autoradiographs from iodinated active and inactive preparations (Fig. 5).

This report describes an immediate relationship between the physiological state of the cell as expressed by shape and a protein component that originates from the cell surface where sizeable amounts can be removed without the aid of enzymes or chelating agents, as in the case of the large molecular weight surface glycoprotein found in untransformed mouse, hamster and chick cells^{15–18}. We have evidence that the mechanism by which this component affects cell shape involves a transmembrane effect on the arrangement of the cell's cytokinetic structures, microfilaments in particular.

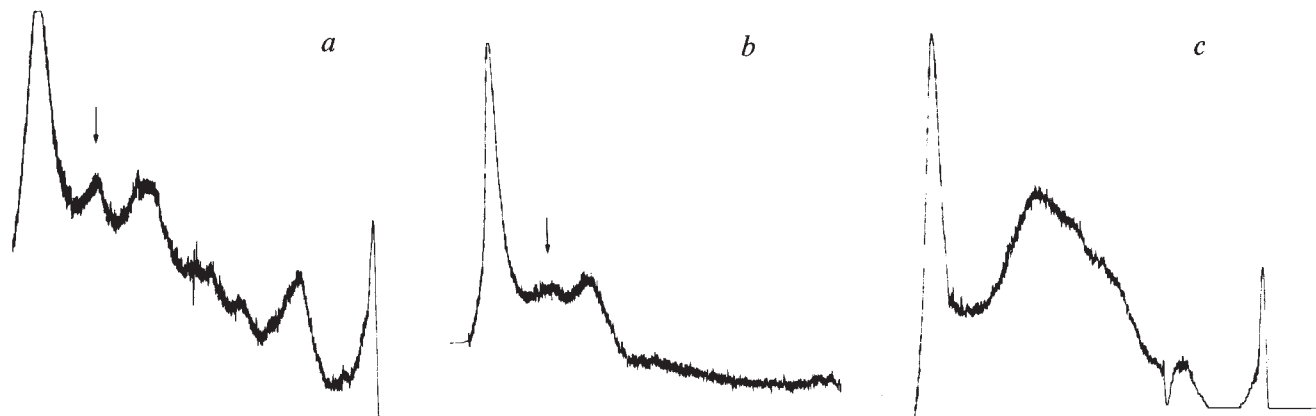
Table 1 Protection of cell rounding by shaken material from normal fibroblasts and from transformed cells

Cell type from which material derived	Cell type on which material tested	
	MEF	3T3
Nil (SFM)	+	+
MEF	–	–
PVTT2	+	±
PVTT3	+	±
L57	+	+
CEF	+	+

PVTT2 and PVTT3, polyoma virus-transformed lines from C57BL/6; L57, spontaneously transformed line of C57BL/6 fibroblasts; CEF, chick embryo fibroblasts. +, rounding; –, protection. All shaken material preparations were derived from the same number of cells.

Figure 6 shows that in the flattened state microfilaments close to the surface membrane have an orderly distribution which is modified to condensed patches near the surface when the rounded state is induced. We have found (unpublished work) that the constituent which affects cell shape is spontaneously released in the cell environment and that the extent and rate of this process is greater in quiescent cells. This indicates that the presence of this component within the surface and its con-

Fig. 5 Densitometric tracings of 7.5% SDS–polyacrylamide gel autoradiographs. *a* Unfractionated shaken material; *b*, active Sephadex fraction; *c*, neighbouring inactive fraction. Arrow indicates peak common to active preparations. Films scanned from right (top of gel) to left. Joyce-Loebl autodensitometer MK III, 0.096 density units per cm.



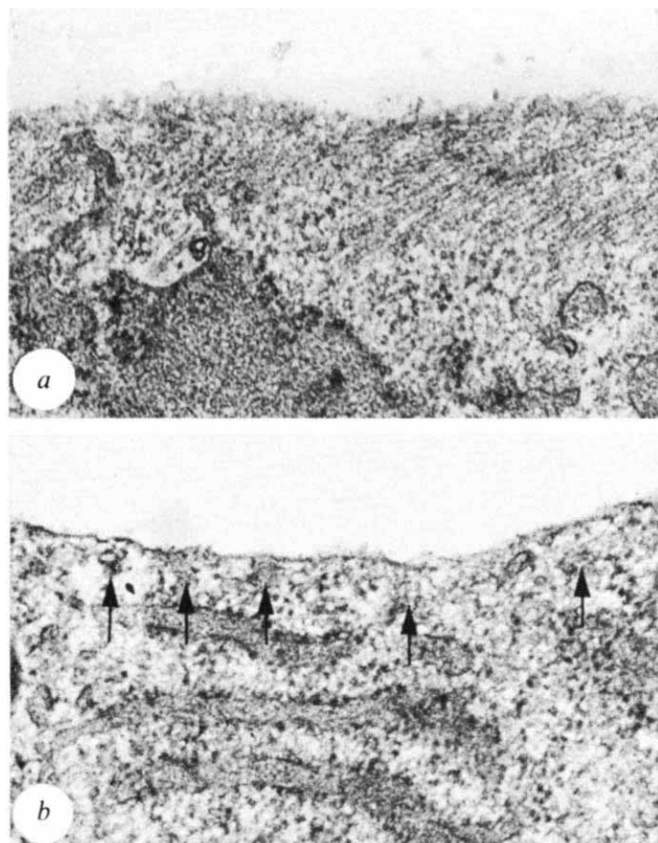


Fig. 6 Electronmicrographs of vertical sections through the upper surface of comparable regions of mouse fibroblasts ($\times 50,000$). *a*, Control cell showing a regular array of microfilaments forming a layer close to the plasma membrane. *b*, Cell 15 min after induction of rounding, showing absence of microfilament layer and appearance of dense aggregations near the plasma membrane (arrows).

centration both at the surface and in the surrounding environment could be an important factor for the determination and modulation of shape during the cell cycle. In a separate report we shall describe its relationship with cytokinetic structures. We have also investigated whether the entire fraction removed by shaking transformed C57BL/6 cell lines¹⁹ in SFM had any effect on the morphology of the normal C57 or 3T3 Swiss fibroblasts, as detectable by prevention or reversion of cell rounding. Table 1 shows that none of these tested materials exerted the marked protective effect shown by the preparation derived from normal fibroblasts. The significance of these results in relation to the presence of a surface factor that affects morphology and its relevance to the phenotypic state and altered growth properties of the cell during transformation will have to be investigated. In view of reports relating cell morphology to concentration of 3', 5'-cyclic AMP^{20,21}, endogenous levels of this nucleotide were assessed in the various experimental conditions described above (courtesy of Dr E. Rozengurt, Imperial Cancer Research Fund Laboratories). The results showed that no detectable differences existed and that the changes of shape were not the consequence of variations in the intracellular concentration of cyclic AMP.

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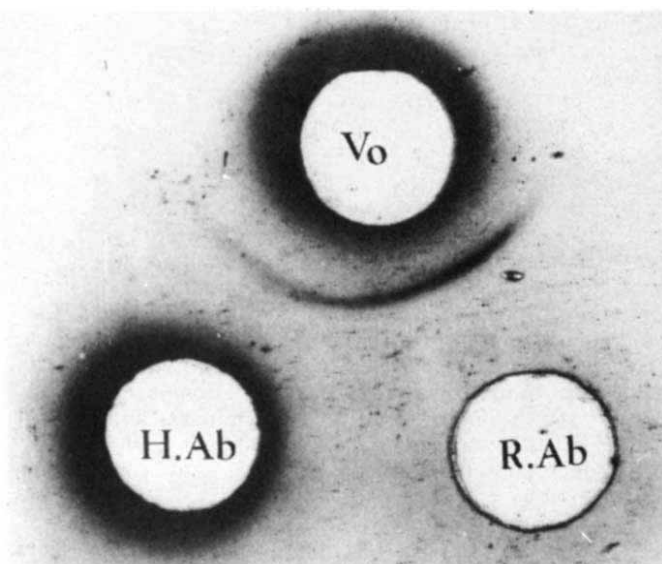
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Precipitating antibodies in von Willebrand's disease

We report the presence of precipitating antibodies in three patients with von Willebrand's disease (VWD), an inherited deficiency of factor VIII (anti-haemophilic factor, AHF). To our knowledge this is the first description of patients with complete biological and immunological deficiency of a plasma-clotting protein responding to replacement therapy by the production of antibodies with properties indistinguishable from those elicited in animals. AHF is a high molecular weight ($> 10^6$) glycoprotein complex¹ associated with two biological activities; one necessary for normal clotting (VIII: C) and the other for normal platelet function (Willebrand factor, VIII: WF). Specific antigenic determinants (VIII: AG) can be reliably quantified using heterologous antisera^{2,3}. Any concept of the structure or the molecular genetics of AHF needs to incorporate the clinical and immunological findings in the two congenital disorders associated with AHF deficiency: haemophilia A and VWD. The former is a sex-linked deficiency of VIII: C alone whereas the latter represents a deficiency of all three parameters of the AHF complex and is inherited as an autosomal trait. Non-precipitating antibodies to AHF, acquired after replacement therapy, are common in haemophilia A (ref. 4) but only one case has been reported in VWD^{5,6}.

Purified IgG was prepared by precipitation with 40% ammonium sulphate and *n*-octanoic acid⁷ from the plasma

Fig. 1 Double diffusion in agarose of purified human AHF complex (Vo) showing a line of identity between homologous antibodies in VWD (H.Ab) and heterologous (rabbit) antibodies (R.Ab).



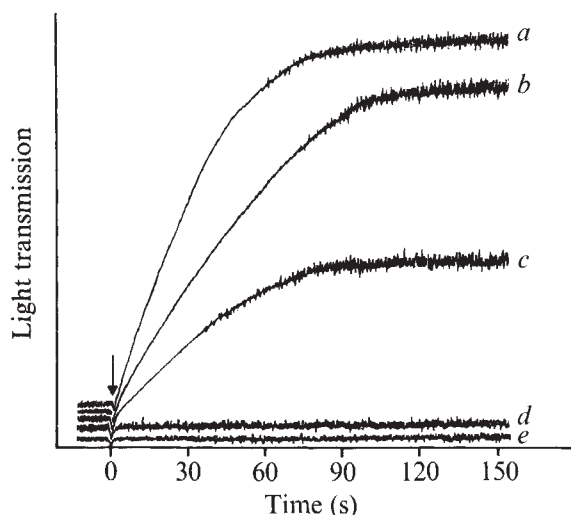


Fig. 2 Inhibition of ristocetin-induced platelet aggregation by plasma from VWD with inhibitor. 50 μ l of patient plasma (serially diluted from 1:1 to 1:8) was mixed with 150 μ l of normal platelet-rich plasma, followed by the addition of ristocetin (1.5 mg ml⁻¹). The control used was 50 μ l of plasma from severe VWD patients with no inhibitor. Similar results were observed with purified IgG from the patients tested using either platelet-rich plasma or normal washed platelets plus normal platelet-poor plasma. *a*, Control; *b*, 1:8; *c*, 1:4; *d*, 1:2; *e*, 1:1.

of three patients with VWD who showed evidence for inhibitor (of 67 tested). The purified IgG in each case gave a line of precipitation with normal and haemophilia A plasma, but not with that of VWD. Precipitation between antibodies and VIII:AG from normal plasma was also demonstrated using the Laurell technique⁸ and by radioimmunoassay⁹. These homologous antibodies therefore have similar properties to those raised in animals against purified human AHF, which is confirmed by the finding of a line of identity between both types of antibodies (Fig. 1). The purified IgG had a low titre (< U ml⁻¹) of anti-VIII:C, whereas they strongly inhibited VIII:WF as measured with ristocetin (Fig. 2). This effect on platelet function was specific for VIII:WF, since there was no inhibition of ADP, collagen or adrenalin-induced platelet aggregation. These IgG partially inhibited bovine and porcine AHF-induced platelet aggregation, however, indicating that the effect was not species specific.

Our findings support the contention that VWD is the result of decreased synthesis of normal AHF (ref. 2). Only those rare patients who are completely deficient would have no immunological tolerance to the protein. Our three patients, two of them brothers, had been transfused on multiple occasions because of an unusual severity of the disease. The absence of measurable plasma levels of VIII:C (ref. 10; < 0.01 U ml⁻¹), VIII:AG (ref. 9; < 2.5 $\times 10^{-4}$ U ml⁻¹) and VIII:WF (ref. 11; < 0.03 U ml⁻¹) and the reduced levels of VIII:AG and VIII:WF in the parents is consistent with the homozygous state^{12,13}. Challenge by transfusion in such patients would be expected to induce an antibody response which, as a result of the multiple antigenic stimulation from a totally foreign protein, could elicit polyvalent antibodies to AHF capable of binding and precipitation. Haemophiliacs seem to synthesise a non-functioning glycoprotein which may have very few antigenic determinants different from normal. Consequently antibodies are more common but precipitation is unlikely to occur because of the paucity of determinants and the difficulty in lattice formation between antigen and antibody.

In conclusion, precipitating antibodies have been found in three polytransfused patients with severe homozygous VWD. Such antibodies seem to be mainly directed towards VIII:WF and VIII:AG rather than towards VIII:C. Since VIII:C may reside on a separate molecule linked by

non-covalent bonds to VIII:AG, it remains to be demonstrated whether the partial neutralisation of VIII:C is specific or secondary to steric hindrance.

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Control of brain tryptophan concentration in rats on a high fat diet

SYNTHESIS in the brain of the neurotransmitter 5-hydroxy-tryptamine (5-HT) is influenced by the concentration of its precursor tryptophan^{1,2}. Much attention has therefore been paid to the determinants of brain tryptophan concentration. Evidence suggests that plasma free tryptophan is an important influence on brain tryptophan^{3,4} and that both concentrations increase when plasma unesterified fatty acids (UFA) rise^{5,6} and release plasma tryptophan from albumin^{7,8}. Other results, however, disagree. Thus feeding rats for 2 h with high fat diets apparently led to large increases in serum UFA and free tryptophan but not brain tryptophan^{9,10,11}. We now present evidence suggesting that the latter findings may have been due to *in vitro* lipolysis during either the preparation of serum or its dialysis at 37 °C for 3.5 h to separate free tryptophan⁹. Here we describe work in which precautions were taken to avoid *in vitro* changes and which is consistent with a relationship between plasma free tryptophan and brain tryptophan concentrations.

Male Sprague-Dawley rats (200-220 g) (Anglia Laboratory Animals) housed three per cage were maintained on ALGH standard rodent diet (Grain Harvesters) and tap water *ad libitum* and subjected to a 6 h:18 h light:dark cycle. After a 5-d adaptation period, food but not water was withdrawn at 1600 and on the following day at 0800 the animals were given access to either their normal diet or to a high fat diet (fatty bacon and bread both fried in corn oil). After 2 h they were decapitated and blood was collected into both heparinised and plain tubes cooled in an ice bath, for the preparation of plasma and serum respectively. Total time between blood collection and separation of plasma and serum (by 5 min centrifugation at 2-4 °C) was 10 and 40 min, respectively. Samples of 0.5 ml of plasma were ultrafiltered for free tryptophan determination as before³, but at 2-4 °C instead of room temperature. Plasma and serum samples (0.1 ml) were also frozen using solid CO₂ immediately after separation for subsequent

determination of UFA¹². Plasma and serum residues were then incubated in a water bath at 37 °C for 4 h, cooled to 2–4 °C and separate plasma samples were ultrafiltered for free tryptophan determination and frozen for determination of UFA as described above. Brains were stored at –20 °C for determination of tryptophan³.

Plasma or serum samples from rats fed on the high fat diet were all lipaemic while those from control fed rats were all clear. UFA concentrations of unincubated plasmas were significantly greater in the former group and the percentage of free tryptophan increased. But as plasma total tryptophan was proportionately lower in the high fat fed rats (see legend to Fig. 1) free tryptophan concentrations of unincubated plasmas were similar in rats on either diet. As Fig. 1a shows, brain tryptophan concentrations were also similar and thus are consistent with the previously found relationship between plasma free tryptophan and brain tryptophan^{3–6}.

When, however, the plasma of rats given the high fat diet was incubated at 37 °C for 4 h, UFA and free tryptophan concentrations increased five- and sevenfold, respectively. (Plasma UFA had already increased more than fourfold in samples withdrawn after 2 h.) In a separate experiment incubation for 4 h did not alter the pH of plasma collected from rats given the high fat diet (before

incubation, $pH=7.75\pm0.05$, $n=3$; after incubation, $pH=7.78\pm0.06$, $n=3$; values shown $\pm$ 1 s.d.). Incubation of plasma from control fed rats led to a relatively small increase in UFA and a negligible change of free tryptophan. The plasma pH of a separate group of control fed rats rose significantly ($P<0.001$) during incubation from 7.78 ± 0.03 ($n=3$) to 8.03 ± 0.03 ($n=3$). Comparison with a standard curve obtained by determining free tryptophan in plasma brought to various pH values with CO₂ showed that this increase of pH would have decreased free tryptophan by about 27%. Even if this is allowed for, it is apparent from Fig. 1 that UFA and free tryptophan both increased massively on incubation of plasmas of fat fed but not control fed animals.

The work of Wurtman's group on free tryptophan and brain tryptophan was carried out not with plasma but with serum^{9–11}. Results in Fig. 1b show that (in agreement with work on human blood¹³) UFA tended to be higher in serum than in plasma prepared from the same blood specimen. The increase was significant in serum from rats given the high fat diet. UFA (and therefore presumably also free tryptophan) continued to increase when the latter serum was incubated at 37 °C.

These results are consistent with many reports indicating *in vitro* lipolysis in plasma^{13–16} especially after intake of fatty food¹⁵, and suggest a reinterpretation of findings previously taken to show lack of effect of large *in vivo* increases of free tryptophan on brain tryptophan in rats fed on fat^{9–11}. Considerable *in vitro* increases could have occurred in this work either during the preparation of serum or subsequently (although in one experiment⁹ major changes were not found after serum incubation).

Our results therefore strengthen previous evidence that plasma free tryptophan is an important determinant of brain tryptophan concentration^{3–6,17}. They do not imply that it is the only physiological influence involved. Insulin secretion, for example, can increase the uptake of tryptophan by the brain, possibly because it decreases plasma concentrations of competing amino acids^{9,18,19}. Recent experiments in which plasma and brain tryptophan were determined after giving either insulin or the diabetogenic drug streptozotocin¹⁷ indicate that plasma free tryptophan and insulin changes may influence brain tryptophan concentration concurrently.

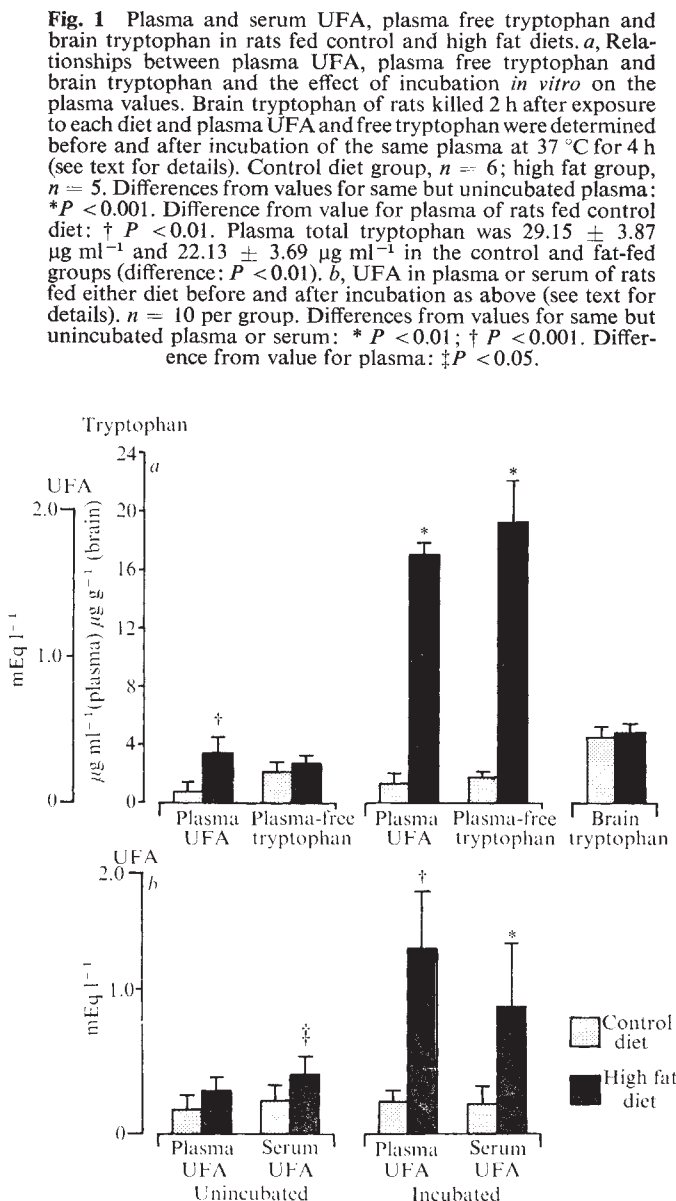
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Inhibition by albumin of tryptophan uptake by rat brain

CHANGES in the tryptophan content of the brain influence the rate of synthesis of brain 5-hydroxytryptamine (5-HT). Therefore, factors that affect the delivery of tryptophan to the brain may affect its 5-HT content. Tryptophan in plasma can be displaced from its binding site on albumin by non-esterified fatty acids (NEFA)¹ and by certain drugs². Although in some circumstances changes in non-albumin bound (NAB) plasma tryptophan, brought about by drugs and changes in plasma NEFA, parallel those of brain tryptophan^{2,3}, this relationship does not apply in all conditions. For example, after administration of glucose plasma NEFA decline, as does NAB tryptophan, but the brain tryptophan content increases⁴. Thus, it is uncertain whether binding of tryptophan to albumin is important in controlling brain tryptophan and hence brain 5-HT. To resolve this controversy we looked at the influence of albumin on the uptake of tryptophan by brain *in vivo*. We found that albumin inhibits tryptophan uptake, and that diffusion, a process that will depend only on the NAB plasma tryptophan concentration, has a small but significant role in the uptake of tryptophan by brain.

Tryptophan uptake by the brain was measured by the technique of Oldendorf⁵. Solutions containing ¹⁴C-tryptophan and ³H₂O (as a freely diffusible standard) were injected into the common carotid artery. After 15 s, when excess solution had been washed out of the microcirculation, the rat was decapitated. The ¹⁴C:³H ratio was determined in the brain and in the injected solution. The ratio for brain, expressed as a percentage of the ratio for the injected solution, is the brain uptake index (BUI).

In agreement with Pardridge and Oldendorf⁶ we found that, as the concentration of tryptophan increased, the BUI decreased. BUI is a measure of the uptake of radioactive tryptophan. As the radioactivity in the solution was constant, the product BUI × (tryptophan concentration) is a measure of the total uptake of tryptophan from the injected solution. Figure 1 is a plot of BUI × (tryptophan concentration) against concentration. The uptake was analysed for conformity to one of two models: (1) active transport combined with non-saturable uptake by diffusion, equation (1); and (2) two active uptake systems with differing affinities, equation (2). The method used to discriminate between these models involves initial evaluation of the constants graphically, based on the double reciprocal plot of uptake against tryptophan concentration in the injected solution, followed by an iterative calculation designed to give the best fit of the line to the observed points⁷.

$$v = \frac{V_s}{K+S} + K_d S \quad (1)$$

$$v = \frac{V_1 S}{K_1 + S} + \frac{V_2 S}{K_2 + S} \quad (2)$$

The fit to equation (1) was judged the more satisfactory on the basis of the low error mean square (0.0221) as compared with equation (2) (3.83). The fit of equation (1) to the experimental points is shown in line (a) of Fig. 1. The values obtained for the constants were $K = 160 \mu\text{M}$, $K_d = 4.51$ and $V = 5,920 \mu\text{M}$. By substituting these values in the two parts of equation (1), lines (b) and (c) were obtained. Note that at 100 μM tryptophan, in the physiological range for that amino acid, diffusion accounts for about 17% of the total uptake. At the concentration of NAB tryptophan found in the normal adult rat—25 μM —the non-saturable mechanism for uptake of the amino acid into the brain accounts for about 12% of the uptake. These data indicate that diffusion of tryptophan across the blood-brain barrier has a small but significant role in

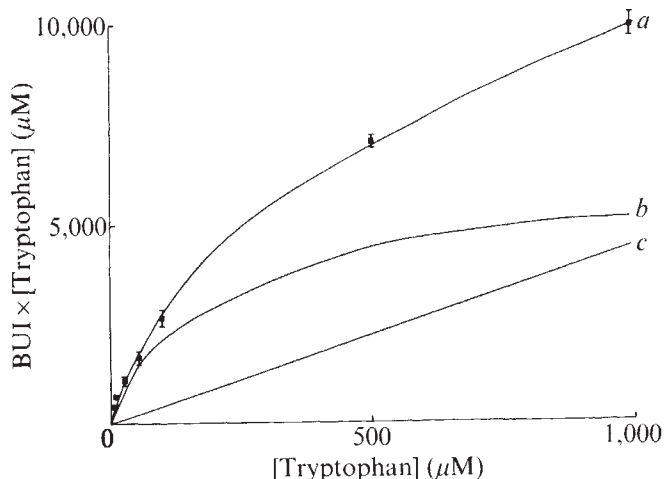


Fig. 1 The variation in tryptophan uptake by brain with various concentrations of tryptophan. A solution containing L-tryptophan, 3 ¹⁴C-side chain (40 mCi mmol⁻¹, New England Nuclear) and ³H₂O (as freely diffusible standard) was injected in the common carotid artery. BUI was measured at the following concentrations of injected tryptophan (TRP) (number of determinations given in parentheses): 6.25 μM (5), 12.5 (13), 25 (20), 50 (13), 100 (13), 500 (3), 1000 (3). The abscissa includes these concentrations. The ordinate represents the amount of tryptophan taken up into the brain during the 15-s perfusion (see text). The points represent means $\pm$ standard error. Line a is drawn as the best fit to the experimental points obtained by methods described in the text. Lines b and c represent the components of line a, corresponding to the saturable and non-saturable processes, respectively.

regulating the brain tryptophan and, consequently, the content of 5HT in the brain, especially after the administration of a tryptophan load.

If the tryptophan is injected in a bolus that contains plasma albumin, the BUI is reduced (Table 1). The addition of albumin to the solution reduced the concentration of tryptophan in free solution from 25 to 6 μM . The BUI for a solution of 6 μM should be, according to the graph in Fig. 1, 70%. But the albumin also reduces the ¹⁴C tryptophan in free solution to the same extent, that is to 24% of its original value. Therefore, the BUI that might be expected if the uptake system acts only on tryptophan in free solution is 24% of 70, or 17. This is close, but not identical, to the actual value found, 22 ± 2 (Table 1). Thus, binding of tryptophan to albumin does inhibit its uptake by the brain, and must have some role in regulating the brain tryptophan content.

The uptake of tyrosine, an amino acid that does not bind to albumin, is unaffected by the presence of that protein in the injected solution (Table 1).

Albumin is not the only component of plasma that affects tryptophan uptake. Some amino acids inhibit the process by competing for the same transport system⁸. But this inhibition has been studied so far only with amino acid concentrations beyond the physiological range. Table 2 shows that when the

Table 1 Effect of albumin on tryptophan and tyrosine uptake by brain

Compound	Albumin	% Amino acid in free solution	BUI*
Tryptophan	—	100	40 $\pm$ 3 (8)
	+	24	22 $\pm$ 2 (9)
Tyrosine	—	100	30 $\pm$ 3 (6)
	+	100	26 $\pm$ 2 (6)

*BUI was determined as described in Fig. 1 using a tryptophan solution of 25 μM and L-tyrosine (¹⁴C-U, 400 mCi mmol⁻¹, New England Nuclear) at 100 μM . Rat albumin (Sigma) was used at a concentration of 60 mg ml⁻¹. The fraction of tryptophan in free solution was determined by ultrafiltration using an Amicon propellant-pressurised ultrafiltration cell with UM 10 membranes (nominal molecular weight cutoff 10,000).

Table 2 The effect of amino acids on tryptophan uptake by brain

Amino acid	Concentration of amino acids*(μ M)				Non-competing
	Control	Low	Mean	High	
Histidine	55	63	70		
Isoleucine	73	101	128		
Leucine	167	203	238		
Methionine	58	73	64		
Phenylalanine	70	83	96		
Tyrosine	96	123	151		
Valine	194	228	262		
Alanine					659
Glycine					302
Lysine					397
Proline					374
Threonine					370
BUI†	41 $\pm$ 2 (7)	26 $\pm$ 5 (6)	21 $\pm$ 2 (6)	13 $\pm$ 1 (6)	37 $\pm$ 4 (5)

*Concentrations used for competing amino acids: the upper and lower limits of the physiological range as well as the mean⁸, for non-competing amino acids: the mean of the physiological range for the rat.

†For a concentration of 25 μ M tryptophan in the injected solution.

concentrations of a group of competing amino acids are raised from the lower to the upper limit of their physiological range there is considerable inhibition of tryptophan uptake. Thus, competition between amino acids for uptake by the brain is a physiological phenomenon, and it is possible that the increased brain tryptophan that is noted after glucose administration⁴ occurs because of a decline in the plasma concentration of the competing amino acids. Alternatively, there might be a change in the activity of the actual uptake system. This factor is neglected when measurements are made on plasma and brain tryptophan alone, but it can be studied conveniently by the method of Oldendorf⁵. Studies in which uptake is measured as well as brain and plasma tryptophan content should elucidate fully the influences the components of plasma on brain tryptophan and 5-HT.

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Monitoring of transmitter metabolites by voltammetry in cerebrospinal fluid following neural pathway stimulation

AN *in vivo* voltammetry technique has been developed which enables direct and continuous measurement to be made of the release of neurotransmitter metabolites in cerebrospinal fluid (CSF) after electrical stimulation or pharmacological manipulations of neural pathways. The measurement is very simple in principle. A potential is applied to a micro carbon electrode stereotactically placed in a lateral ventricle. One then measures the minute current which results from the oxidation or reduction of small molecular weight constituents near the sensor electrode. The potential at which the electrolysis occurs serves as

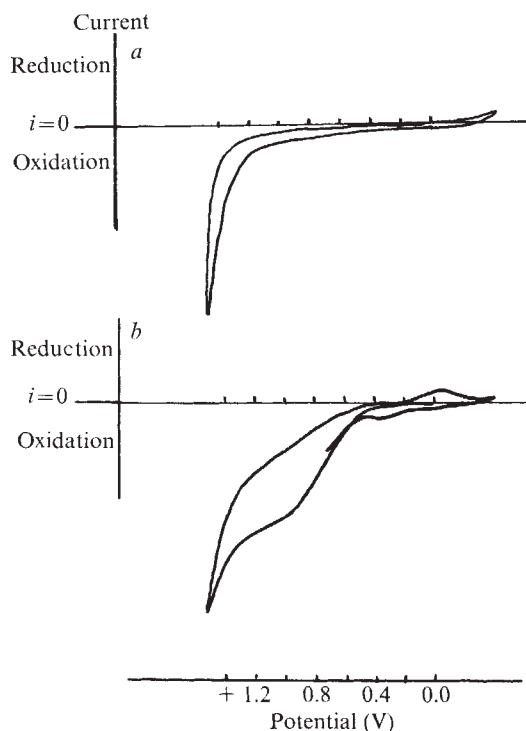
a qualitative indication of the constituent being monitored and the current itself is directly proportional to the concentration of this component in CSF.

The catecholamines and all their metabolites, as well as 5-hydroxytryptamine and its metabolites, are readily oxidised. Thus, the technique is applicable to catecholaminergic and serotonergic neurotransmitter systems. Acetylcholine, GABA and various other aliphatic amines and acids which have suspected neurotransmitter roles are not electroactive and are not sensed by the electrode in the conditions used. We have previously described the *in vivo* voltammetry method especially for examining drugs injected directly into brain tissue^{1,2}. We believe the present application is particularly significant because it provides continuous readout of CSF metabolites following stimulation of neural pathways without the necessity for perfusion or radioactive labelling.

In one test of the technique, we examined the metabolites transferred to the CSF following electrical stimulation of a well-established neural substrate, the dopaminergic nigrostriatal pathway. Rats were implanted with conventional bipolar stimulating electrodes in the pars compacta of the substantia nigra. At the same time, one or both lateral ventricles were implanted with a small guide cannula. Following several days of surgical recovery, the rat was anaesthetised and placed in the head holder of a stereotaxic apparatus. A micro carbon electrode was inserted in the cannula to a depth which ensured contact with the CSF of the lateral ventricle. It is well known that electrical stimulation of the substantia nigra causes a release into the CSF of the dopamine metabolite homovanillic acid (HVA)^{3,4}. The latter was the sought-after electroactive component in these experiments.

Figure 1 illustrates the qualitative aspects of the electrochemical experiment. The cyclic current-voltage curves (voltammograms) of Figs 1a and b were initiated by a triangular wave potential sweep from 0.0 to 1.4 V and then returned to -0.2 V. (All potentials are measured against a micro saturated calomel electrode (SCE) normally placed in contact with brain tissue over the frontal sinus area.) Figure 1a shows the baseline level of com-

Fig. 1 Electrochemical signals in CSF at micro carbon electrode. a, Before stimulation; b, after stimulation.



ponents in the CSF which can be oxidised within this potential range (primarily ascorbate and micromolar levels of HVA). At the current sensitivity used, very little oxidation current is seen in Fig. 1a in these "resting" conditions. Following stimulation of the substantia nigra, Fig. 1b shows a large current with a plateau at around +0.8 V which is a result of HVA oxidation. (Although this is a large increase, all electrochemical currents referred to herein are at most several hundred nA.) The small peaks around +0.1 V in Fig. 1b come from oxidation and reduction of dihydroxyphenylacetic acid (DOPAC) and its *o*-quinone. It is well established that DOPAC is formed during the electrolysis of HVA^{5,6}, and the small peaks serve as an additional electrochemical "marker" for the presence of HVA.

The quantitative measurements are carried out by fixing the potential at +0.8 V and monitoring the current due to HVA oxidation as a function of time. Before stimulation only the low baseline signal illustrated in Fig. 1a is monitored at +0.8 V. The substantia nigra was then stimulated for 20 0.5-s intervals using simple 60-Hz sine waves. In typical experiments, the baseline remained relatively constant for some 15–20 min after stimulation. There then was a sharp rise in HVA signal, followed by a more gradual disappearance. Often such stimulations could be repeated several times and Fig. 2 shows a typical result of two successive stimulations. Successful experiments were carried out with 12 of 22 rats stimulated. Because of difficulties with cannula placement in rat ventricles (our electrodes at present require cannulae of around 1 mm external diameter), we have recently repeated this type of experiment in rabbits. To date, four successful substantia nigra stimulations have been carried out with rabbits. It should be emphasised that no optimisation of stimulation parameters, anaesthetics, electrode size and so on has yet been investigated. A full report of the experimental details is in preparation.

Similar electrical stimulation was carried out in the region of the midline raphe nuclei with rabbits. As expected, an efflux of 5-hydroxyindole acetic acid (5-HIAA) into the CSF was observed by the voltammetric monitoring, but little or no change in HVA.

Pharmacological manipulations of neural substrates can also be monitored by this technique. For example, a large increase in HVA signal in ventricular CSF was observed

some 30 min after an intraperitoneal injection of L-dopa in the rabbit.

Since this type of electrochemical measurement is unfamiliar in electrophysiological studies, we expected its authenticity to be quite properly open to question. Thus, electrochemical measurements were routinely backed up by independent and unequivocal chemical identification of HVA, DOPAC or 5-HIAA by high performance liquid chromatography. When a successful stimulation or drug manipulation was obtained—as evidenced by a current rise at the sensing electrode—we lifted out the electrode and removed 2–5 ml of CSF with a Hamilton syringe. The CSF sample was injected directly into the injection port of a liquid chromatograph. The metabolites were directly analysed by high performance liquid chromatography on a DuPont SAX anion exchange column with a sensitive electrochemical detection system⁷. The CSF metabolites were identical with authentic samples injected into the column and quantitative correlations were possible.

We believe that the present technique will offer distinct advantages over existing methods for examining metabolite changes in CSF following neural pathway manipulation. It is the only technique we know making use of an on-line sensor that provides instantaneous and continuous monitoring of CSF metabolite levels (electrochemical response times are variable, but routinely are 10–20 ms). It involves minimal disturbance of the physiological milieu; electrical currents are always in the nA range and natural CSF and its flow patterns are used rather than perfusion. It is, of course, limited in its scope to the oxidisable neurotransmitter systems and their metabolites, as mentioned earlier. Although the present results are very promising, improvements in electrode miniaturisation, sensitivity and selectivity are desirable. We are now examining modifications to extend the technique to direct tissue measurements with neuronal pathway manipulation.

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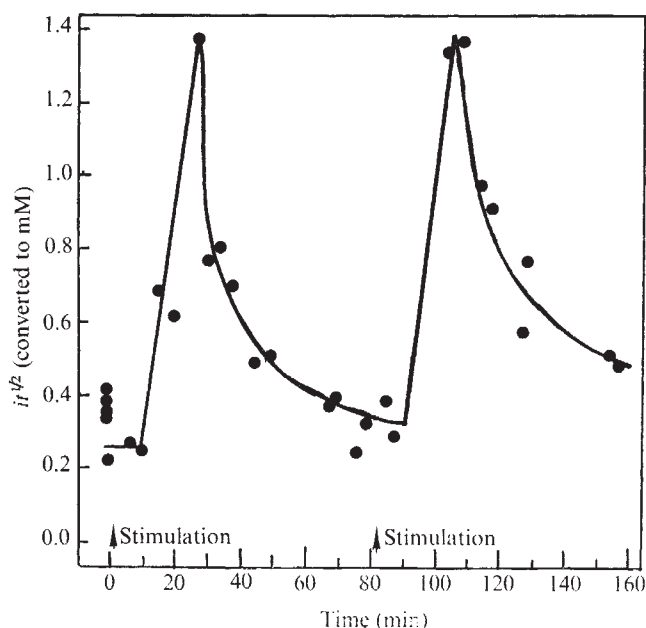
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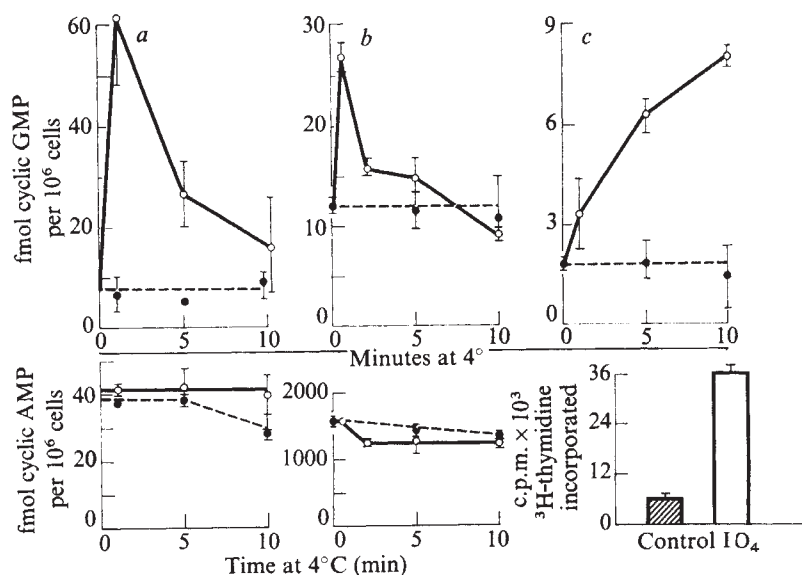
Fig. 2 HVA current response in CSF following substantia nigra stimulation. Current-time product for 200-ms electrolysis was converted to mM concentrations of HVA by external calibrations.



Periodate-induced increase in cyclic GMP in mouse and guinea pig splenic cells in association with mitogenesis

INTERACTION of the lectins phytohaemagglutinin (PHA) and concanavalin A (con A) with the lymphocyte plasma membrane initiates a sequence of metabolic events leading to cell proliferation. The numerous alterations in cellular components and processes that occur early after mitogenic stimulation include enhanced uptake of nucleotides², amino acids^{3,4}, sugars⁵, Ca²⁺ (ref. 6), and K⁺ (ref. 7), enhanced activity of several membrane enzymes^{8,9}, accelerated turnover of phospholipids^{10,11}, increased membrane fluidity¹² and an increased accumulation of cellular cyclic 3',5'-guanosine monophosphate (cyclic GMP)^{13–15}. Demonstrations that exogenous cyclic GMP can stimulate definable functions in lymphocytes^{16,17}, including proliferation¹⁸, and

Fig. 1 Cyclic GMP and cyclic AMP levels and ^3H -thymidine incorporation after exposure of mouse splenic cells to IO_4 . Splens, removed from cervically dislocated 6–8-week-old BALB/c mice, were homogenised gently in PBS. After sedimentation of connective tissue the suspended cells were removed, washed with PBS, and suspended at 20×10^6 cells per ml PBS. The IO_4 , freshly dissolved in PBS, was introduced to achieve a final concentration of 5 mM while the cell suspension was maintained at 4°C . Samples were collected for determination of cyclic nucleotides by adding perchloric acid to a final concentration of 1N. Acid extracts were purified by alumina and AG1X8 column chromatography, and assayed by radioimmunoassay²⁸ after conversion to the 2'-O-acetyl derivative²⁹. Cells in which the proliferative response was monitored were exposed to the IO_4 for 30 min, washed twice with PBS, and suspended at 2×10^6 cell per ml in RPMI 1640 containing foetal calf serum (5%), penicillin ($110 \mu\text{g ml}^{-1}$) and streptomycin ($100 \mu\text{g ml}^{-1}$). The cells were incubated in 5% CO_2 at 37°C for 48 h before a terminal pulse with $1 \mu\text{Ci } ^3\text{H}$ thymidine for 3 h. Cultures were collected by a multiple automatic sample harvester and thymidine incorporation was determined by liquid scintillation spectrometry. The broken lines represent controls.



that there is an association between phyto mitogen action and cyclic GMP accumulation suggest that this nucleotide is a positive effector of proliferation. To investigate further how cyclic GMP may be involved in this sequence of events, we have studied the effects that the oxidant periodate (IO_4) and the reducing agent cysteine have on the cyclic nucleotide levels of rodent splenic cells¹. IO_4 stimulates lymphocyte proliferation¹⁹ and because the oxidant produces definable chemical alterations in membrane components, which can be prevented by reducing agents²⁰, studies with these agents may provide insights into the regulation of cyclic GMP metabolism in relation to mitogenic stimulation.

Figure 1 shows the effect of IO_4 on the concentrations of the cyclic nucleotide in suspensions of mouse splenic cells, maintained at 4°C , in three experiments. The concentrations of cyclic GMP in the cells not exposed to the mitogen varied from animal to animal, ranging from 2.5 to $12.5 \text{ fmol per } 10^6$ cells. In spite of the variation, treatment with IO_4 consistently caused an enhanced accumulation of cyclic GMP but did not alter the concentration of cyclic 3',5'-adenosine monophosphate (cyclic AMP). The increase in cyclic GMP concentration ranged from two- to tenfold and the temporal characteristics of the enhanced accumulation differed in each experiment shown. The IO_4 -induced increases in cyclic GMP were accompanied by a stimulation of lymphocyte proliferation as illustrated by the increased amount of ^3H -thymidine incorporated into cells cultured after exposure to IO_4 (Fig. 1c).

The cyclic nucleotides in guinea pig splenic cells responded similarly when exposed to IO_4 ; cellular levels of cyclic GMP increased but cyclic AMP was unaffected (Fig. 2). The addition of CaCl_2 (0.2 mM) to the cation-free phosphate-buffered saline media (PBS) did not enhance the ability of IO_4 to promote accumulation of cyclic GMP.

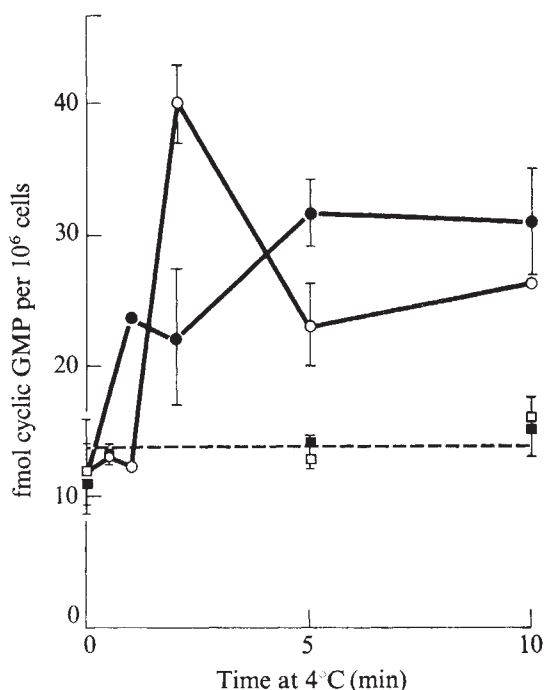
The reducing agent cysteine prevented the IO_4 -promoted increase in cyclic GMP. Cysteine alone or together with IO_4 significantly lowered (50%) the concentration of the cyclic nucleotide (Fig. 3). The increase in ^3H -thymidine incorporation seen after exposure to IO_4 alone was prevented in the cells exposed to IO_4 in combination with cysteine. There was no change in the cyclic AMP content after the addition of either the oxidising or the reducing agent.

Thirteen experiments were conducted to examine the effects of IO_4 on cyclic GMP levels. In the nine experiments in which an increase in cyclic GMP was detectable (70–1,000%) there was a concomitant increase in the rate of ^3H -thymidine incorporation. But in the other four experi-

ments there was no measurable increase in either cyclic GMP or in ^3H -thymidine incorporation, indicating that IO_4 had no oxidative influence in these experiments.

The increases in cyclic GMP detectable after exposure to IO_4 were smaller than those reported after stimulation of human or rodent peripheral blood lymphocytes with PHA or con A^{13,14}. This may reflect the difference between the cell populations studied or a difference in the nature of the mitogenic stimulus. In the conditions of mitogenic stimulation in our experiments, however, the changes occurred at 4°C , which may have minimised the increases. Furthermore, splenic cell suspensions, although consisting primarily of lymphocytes, contain other cells such as polymorphonuclear leukocytes and macrophages. It is not possible to determine from the experiments reported here

Fig. 2 Effect of IO_4 on cyclic GMP of guinea pig splenic cells in the presence and absence of Ca^{2+} . Cell suspensions were prepared from guinea pigs (250–350 g) as described for Fig. 1. CaCl_2 (0.2 mM) was added to the cells suspended in PBS at 4°C 10 min before addition of IO_4 (5 mM). ●, Plus Ca^{2+} ; ○, without Ca^{2+} ; ■, control plus Ca^{2+} ; □, control without Ca^{2+} .



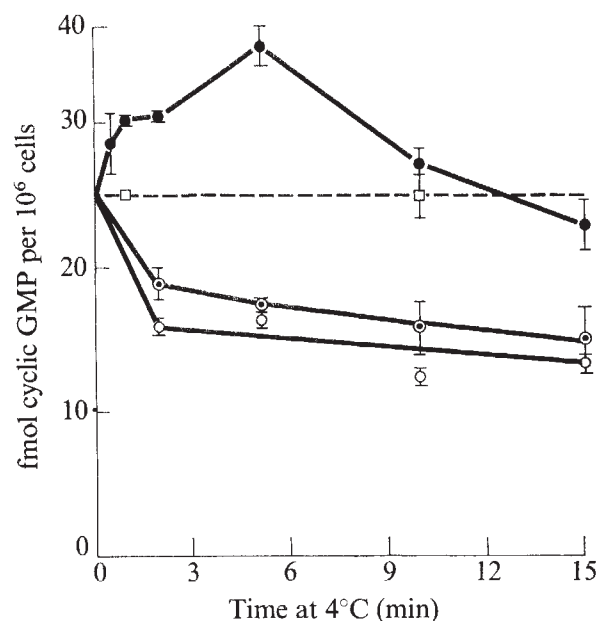


Fig. 3 Effect of cysteine on basal (○) and IO_4 -stimulated (○●) cyclic GMP of mouse splenic cells. All methods were as described in Fig. 1. Cysteine (1 mM) was added to the cells just before addition of the IO_4 (1 mM). ●, Plus IO_4 alone; □, control.

whether the increases in cyclic GMP derived from an alteration induced in lymphocytes directly or secondarily, or whether they reflect an influence on another cell type. The importance of this question increases in the light of demonstrations of a requirement for macrophages for IO_4 stimulation of lymphocytes²¹ and of an involvement of cyclic GMP in the release of lymphocyte-stimulating factors by macrophages²².

The ability of IO_4 to stimulate an increase in cyclic GMP suggests that an alteration of the cell membrane involving oxidation of a specific constituent, such as the sialic acid moiety indicated by the work of Novogrodsky²⁰, is coupled to a system involved in regulating guanylate cyclase. The reciprocal effects of IO_4 and cysteine on cyclic GMP suggest a mechanism for regulating guanylate cyclase activity through cellular events involving oxidation and reduction. This concept is supported by observations that soluble fractions of guanylate cyclase from platelets²³, and lung²⁴, as well as from lymphocytes (M. K. H., J. H. S. and N. D. G., unpublished observation), undergo a time dependent, O_2 -requiring "autoactivation" which is inhibitable by the reducing agent dithiothreitol. Such a mechanism may be allied to that by which oxidation and reduction have been suggested to underlie the modulation of cell proliferation²⁷.

It is not known whether all the metabolic changes occurring early after mitogenic stimulation of lymphocytes are necessary for DNA synthesis; whether some, although not absolutely required, serve to modulate the rate of proliferation, or whether some are merely related to the generalised changes in the membrane. The number and variety of early events suggest that no single event signals commitment to proliferation.

Agents such as acetylcholine and imidazole can increase cyclic GMP in lymphocytes without promoting proliferation although they potentiate the action of phytomitogens^{25,26}. Therefore, the increased cellular accumulation of this cyclic nucleotide is probably not the sole initiator of proliferation, but rather, acts in parallel or in sequence with the other metabolic changes resulting from mitogenic action.

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Evidence for non-deoxynucleotide linkers in Ehrlich ascites tumour cell DNA

ALTHOUGH many authors have used lysis of eukaryotic cells on alkaline sucrose gradients to measure the size of single-stranded DNA subunits, these studies cannot distinguish a true DNA subunit from a subunit containing non-DNA, alkali-stable linkers. We report here that when Ehrlich ascites tumour (EAT) cell DNA is treated with Pronase before alkali denaturation the size of the released subunit changes from $>70S$ to $26S$. T7 DNA present throughout remains unchanged in size, indicating that the change is not due to a soluble nonspecific nuclease.

Kavenoff and Zimm¹ have shown that the double-stranded DNA from *Drosophila* cells seems to be continuous throughout the length of the chromosome. These results do not, however, rule out the possibility of single-stranded nicks or non-deoxynucleotide linkers². When eukaryotic DNA is lysed in alkali and measured either in alkaline sucrose gradients³⁻⁶ or by viscoelastic techniques⁷⁻⁸, it seems to have a molecular weight of 1×10^8 - 2×10^8 or larger. Although there is some disagreement, most authors consider this material to represent single-stranded DNA, particularly if the DNA is allowed to stand in alkaline detergent solutions for 3-6 h^{4,5,7}.

We also observe that nearly all the DNA of EAT cells (G ϕ strain)⁹ sediments faster than T4 (not shown) or T7 marker DNAs in alkaline sucrose gradients (Fig. 1a). This DNA has a molecular weight of 0.9×10^8 - 1.2×10^8 (not

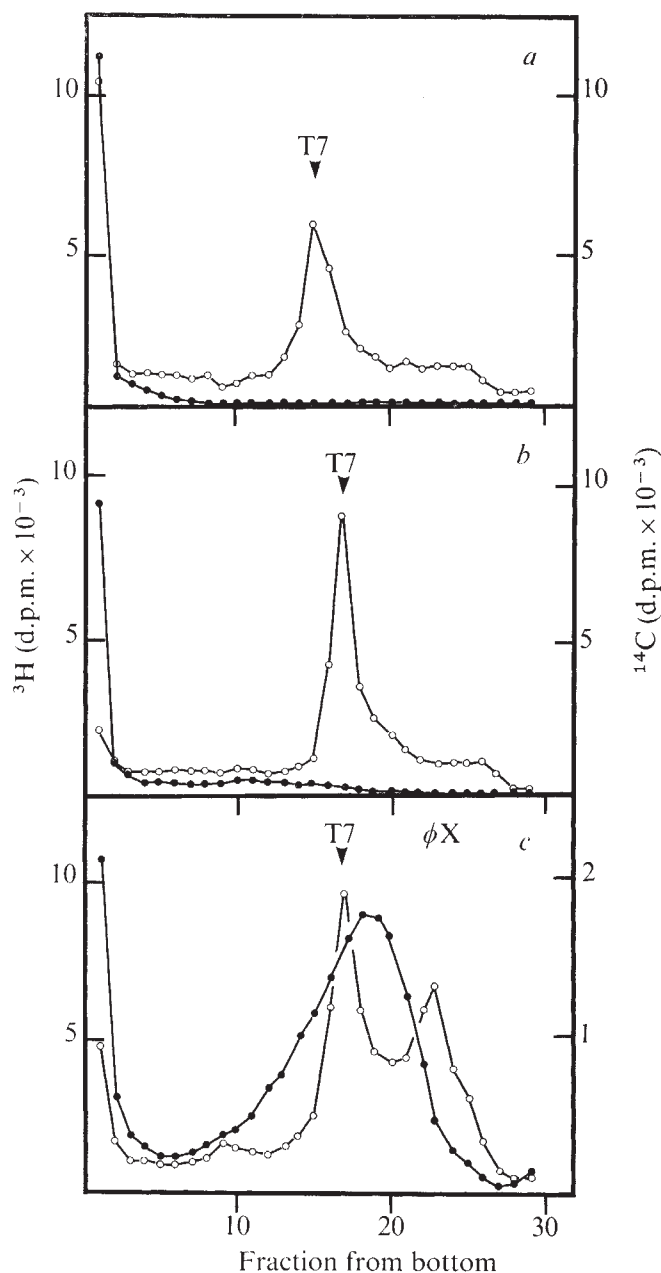


Fig. 1 Ehrlich ascites tumour cells were labelled by intraperitoneal injection of 5 μ Ci 14 C-thymidine (Amersham-Searle). The next day the cells were collected, washed twice by centrifugation in Hanks balanced salt solution, and suspended in 0.01 M EDTA, pH 7.5, at 10^7 cells per ml. A cell suspension (0.5 ml) was added to 0.5 ml of lysis buffer at 50 °C in polyallomer SW 27 centrifuge tubes. The final concentrations in the lysate were 0.5 M NaCl, 0.05 M EDTA, pH 7.5, 0.3% sarkosyl detergent. 3 H-labelled T7 and ϕ X phage markers and pretreated (10 min at 80 °C, 1 h autodigestion at 37 °C) Pronase P (3 mg ml $^{-1}$, Serva), when included, were present before addition of the cell suspension. Lysis was instantaneous. *a*, NaOH (0.1 ml of 1N) was added before cell lysis. After this alkaline lysis (pH 12), the lysate was at 20 °C for 9 h before gradient formation. 3 H-T7 was present throughout. *b*, Cells lysed at 50 °C in buffer without Pronase and incubated at 50 °C for 5 h, followed by addition of 0.1 ml 1N NaOH for 4 h. 3 H-T7 was present throughout. *c*, Cells lysed at 50 °C in buffer with Pronase and incubated at 50 °C for 5 h, followed by addition of 0.1 ml 1N NaOH. 3 H-T7 and ϕ X were present throughout. 5–20% alkaline sucrose gradients containing 0.2 M NaCl, 0.3 N NaOH, 0.05 M EDTA, and 0.3% sarkosyl were formed beneath the lysate. Centrifugation was at 25,000 r.p.m., 4 °C, for 12 h. Gradients were pumped from the bottom, fractions precipitated on Millipore filters with 10% trichloroacetic acid, washed with TCA and ethanol, and counted. ●, 14 C-labelled cell DNA; ○, 3 H-labelled phage DNA.

shown) after alkaline lysis for 3–6 h at a pH which is sufficient to denature DNA completely 4,7 and to dissociate all but the most tightly bound material. In agreement with previous research 6 , very little cell protein cosediments with the rapidly sedimenting material. Indeed, only three protein bands are observed electrophoretically after protein specific iodination of this material (U. Plagens and D. Werner, unpublished). Shear was minimised by lysis of the cells and markers in polyallomer centrifuge tubes with subsequent formation of the sucrose gradient beneath the sample. If the cells were lysed initially in a neutral lysing solution and only made alkaline after 3–6 h at 50 °C, the DNA still sediments faster than 70S (Fig. 1*b*). Similar results are obtained at lower temperature, lower salt, less EDTA and sodium dodecyl sulphate (SDS) instead of sarkosyl. But if Pronase (3 mg ml $^{-1}$, heated to 80 °C for 10 min, autodigested at 37 °C for 60 min and exhibiting no nuclease activity on phage DNA) is present in the neutral lysing conditions, more than 80% of the single-stranded DNA released by alkali now sediments at 26–35S (6×10^6 – 12×10^6 daltons) (Fig. 1*c*). This is more than an order of magnitude smaller than the smallest subunits observed after lysis of cells directly on alkaline sucrose gradients $^{3-6}$. After exhaustive digestion with Pronase, the peak reaches a limit at 26S. With less extensive digestion, the peak is broader and closer to 35S. In each case, T7 marker DNA was present throughout the lysis and incubation of the cells. The marker remained apparently unchanged, giving an initial indication of the absence of substantial soluble DNase activity, either in the presence or absence of Pronase. Moreover, only in the case of immediate lysis in alkali is there any evidence of substantial entrapment of T7-sized DNA with the cellular DNA sedimenting to the bottom. Thus, there is no evidence that the rapidly sedimenting DNA observed in the absence of Pronase is a nonspecific aggregate of 26S subunits.

To more explicitly detect any DNase activity in, or activated by, the addition of Pronase, the experiment described in Fig. 2 was performed. 3 H-T7 was present during the neutral lysis of cells in the presence of Pronase and subsequent alkaline denaturation and 32 P-T7 was then added to the alkaline lysate (Fig. 2*a*). The label present throughout lysis and the label added only after alkaline denaturation cosediment. Alkaline lysis of the phage in the absence of cells and Pronase gives similar patterns (Fig. 2*b*) as does digestion of phage DNA with Pronase in the absence of cell DNA. The small excess of more slowly sedimenting material in the 3 H-labelled phage is also observed if the order of addition of phage is reversed. Similar lack of nuclease activity is observed for ϕ X and λ DNA markers. It is thus unlikely that the change observed in the size of alkaline denatured EAT DNA after digestion with Pronase is due to a soluble DNase, since such a DNase activity must be able efficiently to recognise a site present in EAT DNA but not in any of the phage DNAs tested and to show this specificity in high salt, the absence of divalent ions, and detergent at 50 °C.

There are two general explanations for these results. EAT chromatin may contain a tightly-bound nuclease which is activated by the Pronase treatment. Such a tightly-bound nuclease activated by Pronase, SDS or heat has been described associated with colE1 plasmid DNA 10 . Alternatively, EAT DNA may contain a non-deoxynucleotide linker which is cleaved (or made alkali labile) by the Pronase treatment. Possible candidates for such a linker might include: (1) a modified, alkali-resistant ribonucleotide, such as a 2'-O-Me ribonucleotide, which is susceptible to an RNase activity present in, or activated by, the Pronase; (2) an RNA linker protected from alkaline degradation by a protein, either covalently linked or tightly-bound, and thus made susceptible to alkaline lysis after Pronase digestion, and (3) a protein or peptide linker in the DNA.

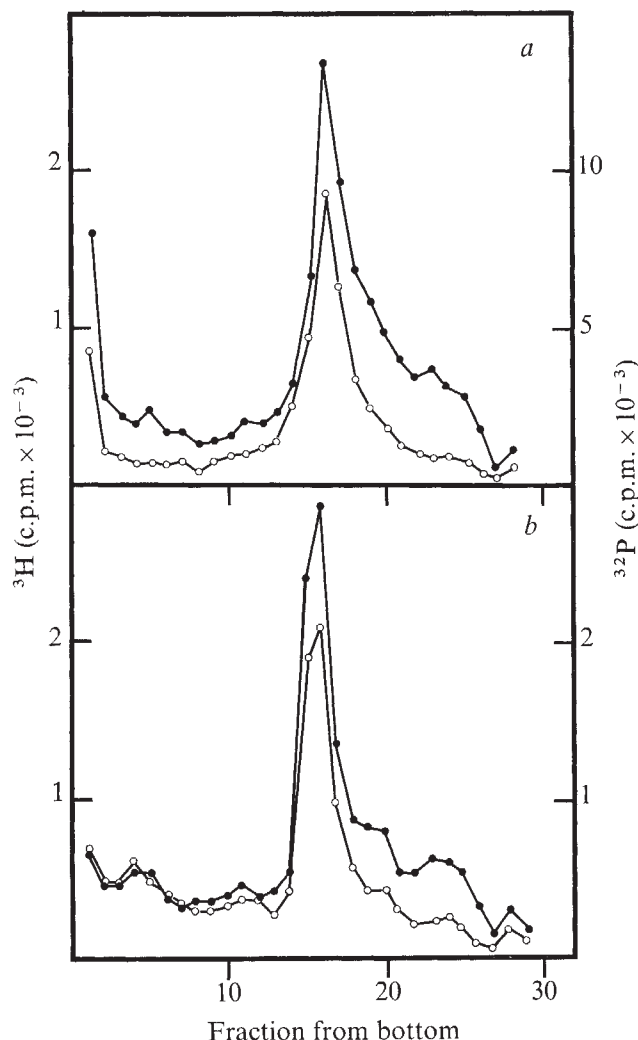


Fig. 2 *a*, 5×10^6 unlabelled Ehrlich ascites tumour cells were lysed in 1 ml of lysis buffer containing Pronase (3 mg ml⁻¹) and ³H-T7 phage marker. The lysate was incubated for 5 h at 50 °C, followed by addition of 0.1 ml of 1N NaOH. After addition of alkali, ³²P-T7 phage was added. *b*, ³H and ³²P-labelled phage were lysed in 1 ml of lysis buffer made alkaline by addition of 0.1 ml of 1N NaOH. Alkaline sucrose gradients were formed, centrifuged, fractionated and counted as described in the legend to Fig. 1. ●, ³H-T7; ○, ³²P-T7.

A preliminary experiment indicates that a chromatin-bound nuclease activated by Pronase is unlikely, since DNA lysed in alkali for 60 min, subsequently neutralised by dialysis, and treated with Pronase also exhibits primarily 26S DNA after a second alkaline lysis. Similarly treated DNA not digested with Pronase is rapidly sedimenting. It is unlikely that a chromatin-bound nuclease would survive the initial alkaline lysis.

RNA linkers have been observed in mitochondrial DNA¹¹, and we cannot, at present, exclude the possibility of a modified alkali-resistant RNA linker or an RNA linker protected from alkali by protein.

A protein or peptide linker should be affected by Pronase digestion. The possibility of protein linkers in eukaryotic chromatin has been indicated by the presence of associated, cosedimenting peptide sequences in purified DNA¹². But the early discussions of protein linkers in DNA often assumed such linkers were "in register" on the anti-parallel strands¹³. Such "in register" linkers are unlikely since double-stranded DNA is insensitive to proteolysis¹. Pronase-treated EAT cell DNA sedimented in neutral conditions is also extremely large if precautions are taken to avoid shear and denaturing conditions. It should be noted, however, that a

protein linker has been observed in adenovirus DNA¹⁴ and is apparently related to its replication.

Since stationary phase cells have been reported to have fragmented DNA of the size observed here¹⁵, it is important to note that the EAT cells had a labelling index of $28 \pm 1\%$. Results similar to those described here have also been obtained with the Karzel strain¹⁶ of EAT cells in exponential growth in tissue culture, using a lower cell density in the lysate.

Using similar lysing conditions with CHO (ref. 3) or mouse L cells¹⁷, others have observed a single-strand subunit of 60×10^6 or 45×10^6 daltons, which is larger than the pieces observed here but smaller than subunits observed by simple alkaline lysis³⁻⁸. Moreover, Taylor *et al.*^{18,19} have observed the double-strand equivalent of the single-stranded 26S DNA in CHO cells. These double-stranded fragments seemed to be enriched in newly-replicated DNA.

Many authors³⁻⁸ have used lysis of eukaryotic cells in alkali to measure the size of long single-stranded DNA. The data presented here indicate that the single-stranded 'DNA' may be composed of smaller subunits linked by alkali stable linkers which can be removed or made alkali labile by treatment with Pronase. The 26S subunits produced do not seem to be a result of soluble nuclease activity during digestion with Pronase.

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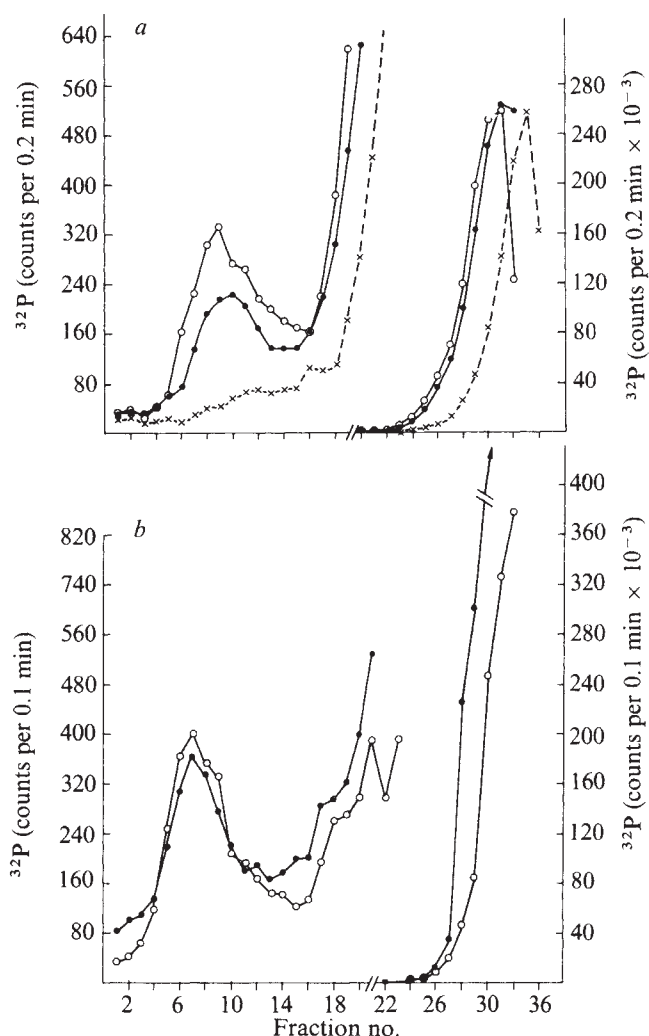
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Intact 3' end of 16S rRNA is not required for specific mRNA binding

INITIATION of protein synthesis in bacteria requires that ribosomes bind to specific regions of messenger RNA (mRNA) in a complex which also contains fMet-tRNA, GTP, initiation factors¹ and involves the ribosomal proteins S1 and S12 (refs 2-5). Shine and Dalgarno proposed a mechanism for initiation of protein synthesis in which certain purine-rich nucleotide sequences near the initiator codon of prokaryotic messages were postulated to form base pairs with the nucleotide sequence (5') ACCUCCUUA(3') near the 3' end of the 16S ribosomal RNA^{6,7}. The formation, *in vitro*, of a complex between the 3' end of the 16S rRNA from *Escherichia coli* ribosomes and the initiator

Fig. 1 Sucrose density gradient analysis of initiation complexes after RNase digestion. Binding reactions were carried out in conditions described by Steitz⁹, at 37 °C in 0.1 ml of a solution containing 0.1 M Tris-HCl (pH 7.4), 0.05 M NH₄Cl; 0.005 M magnesium acetate (RBS buffer); 0.001 M GTP; 3–5 µg of purified *N*-formylmethionyl tRNA from *E. coli*; (α -³²P-UTP)-labelled f1 or ϕ X174 mRNA synthesised *in vitro*, as described by Pieczenik, *et al.*¹⁰, specific activity $\sim 2 \times 10^8$ counts μ g⁻¹; 4.5 A₂₆₀ of *E. coli* ribosomes, and 37.5 µg phage f2 RNA. The binding reactions were done in conditions of limiting ribosomes. This was determined by a set of binding reactions (not shown) with constant amounts of ribosomes and increasing amounts of unlabelled f2 RNA, as competitor for a constant amount of radioactively labelled mRNA. The ribosomes were isolated from S-30 extracts prepared as described by Webster, *et al.*¹⁶ 0.225 ml of S-30 was incubated at 37 °C with TM buffer (50 mM Tris-HCl, pH 7.6; 30 mM NH₄Cl; 10 mM Mg acetate) (control) or with 0.06 ml of a solution of colicin E3, 2.2 mg ml⁻¹. At the end of 20 min, 18 µg of colicin E3 immunity protein¹⁷ in TM buffer were then removed to terminate the action of the colicin. Aliquots were then removed to measure the capacity of the treated ribosomes to carry out f2 RNA-directed protein synthesis *in vitro*¹⁷. Debris was removed from the samples by centrifugation at 12,500 r.p.m. for 15 min and then the ribosomes were pelleted by centrifugation for 3.5 h at 35,000 r.p.m. in the SW50.1 rotor. The supernatants were discarded and the ribosomal pellets suspended in TM buffer to give a final concentration of approximately 1,000 A₂₂₃ ml⁻¹ for the binding reactions. Binding reactions were carried out for 12 min at 37 °C, cooled, and then pancreatic ribonuclease (Worthington) was added to give a final concentration of 5.5 µg ml⁻¹. Reactions were warmed at 37 °C for 30 s, incubated at room temperature for 20 min, cooled on ice, layered on to 5 ml 5–20% sucrose gradients in RBS buffer, centrifuged for 2.25 h at 37,000 r.p.m. in the SW50.1 rotor of the Beckman Model L2-65B ultracentrifuge, at 5 °C. Fractions of 2 drops each were collected through a needle at the bottom of the gradients and their Cerenkov radiation monitored. *a*, Binding of ribosomes to ³²P-f1 mRNA. ●, control ribosomes; ○, colicin E3-treated ribosomes; ×, binding reaction done in the absence of fMet-tRNA. *b*, Binding of ribosomes to ³²P- ϕ X174 mRNA. ●, control ribosomes; ○, colicin E3-treated ribosomes.



region of phage R17 A-protein mRNA has been demonstrated⁸. This binding supports the involvement of such base pairing in the binding of mRNA to ribosomes, at least for this message.

The ability of ribosomes to protect regions of mRNA of specific sequence from nuclease digestion is considered to be a general measure of specific binding between ribosomes and mRNA⁹, and depends on the presence of fMet-tRNA⁹ and yields specific sequences on fingerprint analysis⁹.

Colicin E3 induces a specific break 49 nucleotides from the 3' end of the 16S rRNA in the 30S ribosomal subunit, but does not lead to the loss of the cleavage product from the ribosome^{11–13}. The question arose, given the Shine and Dalgarno hypothesis⁶, as to whether colicin treatment of ribosomes would affect the mRNA binding reaction. We report here that treatment of *E. coli* ribosomes with colicin E3 had no significant effect on their ability to protect phage f1 or ϕ X174 mRNA initiation sequences from RNase digestion (Fig. 1). Furthermore, fingerprinting analysis of the protected RNA suggests that the complexity and specificity of the protected RNA remain largely unchanged following colicin treatment (Fig. 2). The amount of radioactively labelled mRNA protected from RNase digestion was, in fact, consistently higher when colicin-treated ribosomes were used than when untreated ribosomes were used in the protection assay (Fig. 1).

As shown in Fig. 1, the colicin treatment had lowered the ability of the treated ribosomes to carry out f2 RNA-directed protein synthesis by 75%. Even when colicin treatment was carried out in the presence of f2 mRNA and supernatant factors, a procedure which inactivates ribosomes to greater than 90% (ref. 16), little or no decrease in their ability to protect initiation sequences was observed (data not shown).

Ribosomes treated in the presence of colicin E3 immunity protein, which prevents the action of colicin E3 *in vitro*¹⁷,

behaved like untreated, control ribosomes (data not shown).

To determine whether there were any differences in the sequences protected by treated ribosomes, the peaks of ribosome-bound mRNA from the reactions shown in Fig. 1 were collected, separated from the ribosomes, and subjected to two-dimensional T₁ fingerprint analysis, as described by Pieczenik, *et al.*¹⁰. The results for the f1 mRNA are shown in Fig. 2. The f1 mRNA protected by untreated and E3-treated ribosomes give similar fingerprints. There may be some minor differences in the amounts of some of the sequences protected, as measured by the intensity of the spots. No sequences were, however, completely eliminated, nor did any new spots appear as a result of the colicin treatment. The ribosome-protected fragments from ϕ X174 mRNA (Fig. 1) were also subjected to two-dimensional T₁ fingerprint analysis, and here again the sequences protected by colicin-treated ribosomes did not differ appreciably from those protected by the untreated ribosomes (Ravetch, Robertson and Model, unpublished). Thus we infer that there was no change in the specific mRNA sequences protected by those ribosomes.

The three ribosome binding sites of phage f1 mRNA have been sequenced¹⁰. Their sequences have the potential to form base-pair interactions with the polypyrimidine stretch near the 3' end of the 16S rRNA. These potential structures are shown in Fig. 3. Although the number of base pairs differ, the free energies estimated by using the method of Tinoco, *et al.*¹⁸ are comparable. These potential structures can be expected to have similar stabilities.

Although Tai and Davis¹⁹, Tai²⁰, and Konisky²¹ have

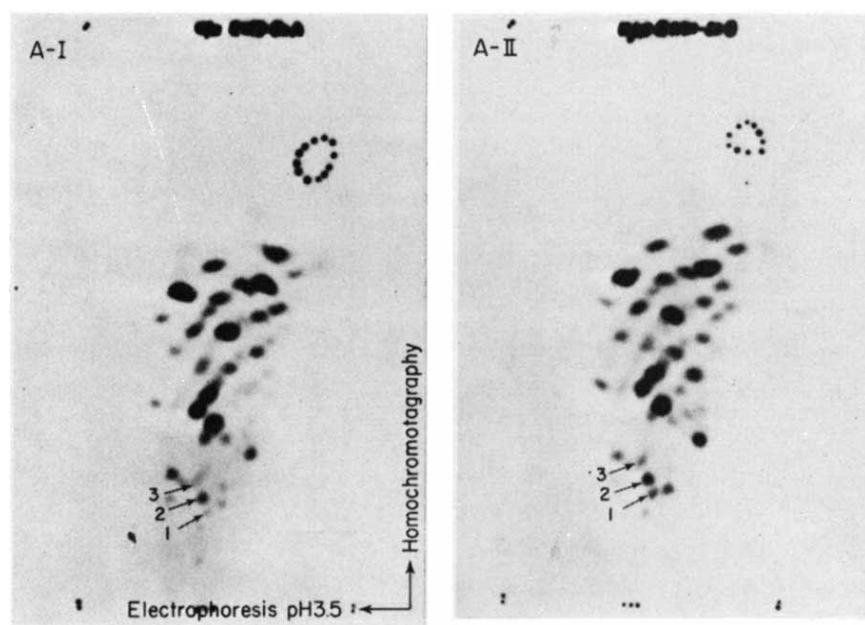


Fig. 2 T_1 fingerprint analysis of ribosome-protected fragments from ^{32}P -labelled f1 mRNA for untreated (A-I) and colicine E3-treated (A-II) ribosomes. Two-dimensional fingerprint analysis was carried out as described previously²⁷. Arrows indicate the major T_1 oligonucleotides of the three ribosome binding sites of f1 mRNA¹⁰. The origins of chromatography were uniformly at the position marked in Fig. A-I.

reported that colicine E3 inhibits initiation of protein synthesis on natural messages, others have reported that colicine E3 treatment does not inhibit (AUG)-dependent binding of fMet-tRNA²² or poly(U) or MS2 RNA²³ to 30S subunits. Our results indicate that colicine E3-induced cleavage of ribosomes does not prevent the formation of 70S initiation complexes on natural messages, so that block which colicine E3 causes in protein synthesis must be at a later step. Colicine treatment in the conditions used here does not result in release of the 3' terminal RNA fragments from the 30S ribosomal subunit^{13,15}, so that while an intact 16S RNA is not required for initiation complex formation, the presence of the cleavage fragment may still be needed for proper alignment of the message on the ribosome.

Baan *et al.*²⁴ have found that cloacin DF13, which has a mode of action virtually identical to colicine E3 (ref. 25), does not appreciably inhibit the formation of an initiation complex between ribosomes and MS2 mRNA in the absence of IF-1. They find that cleavage by the cloacin causes a reduction in the rate, but not the final amount of initiation complex formed, and they postulate improper functioning of IF-1. The initiation complexes formed were fully reactive with puromycin. They also observed a reduced ability

of the second aminoacyl tRNA (alanyl-tRNA for the case of MS2 RNA) to bind, but such alanyl-tRNA as did bind was able to participate in dipeptide formation. Their observations are in accord with our results. Our data and those described above²⁴, therefore, bear on the question as to whether colicine E3 treatment of ribosomes inhibits the initiation or elongation steps of protein synthesis. Since the peptidyl transferase has previously been shown to be undamaged by colicine E3 treatment²², and dipeptide formation is not blocked²⁴, the lesion induced by colicine E3 is most likely in the translocation step of protein synthesis.

Our results suggest that cleavage of the 3' end of the 16S ribosomal RNA does not affect the specificity of the binding of ribosomes to f1 or ϕ X174 mRNA. An intact 3' end of the 16S rRNA is apparently not essential to confer stability and specificity on mRNA binding. In fact, perhaps the greater flexibility of the 16S rRNA allowed by its cleavage by colicine E3 may be responsible for the enhanced level of binding observed after colicine E3 treatment (Fig. 1). The failure of colicine E3-treated ribosomes to function in f2 mRNA-directed protein synthesis implies, however, that an intact 16S rRNA is required for translocation to occur on the ribosomes.

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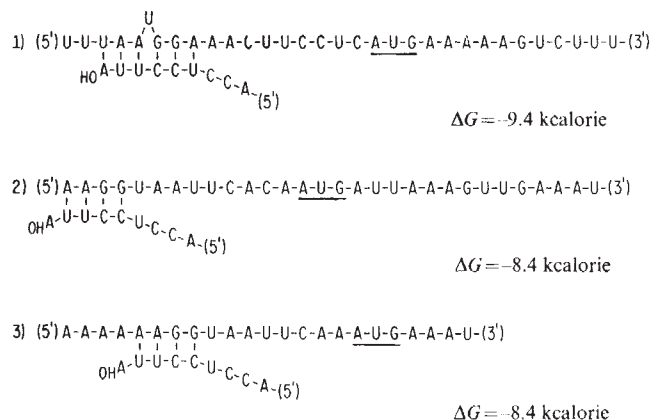
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Fig. 3 Potential base-pair interactions between f1 mRNA ribosome binding sites and the 3' end of 16S ribosomal RNA. Sequence determination was described by Piecznik *et al.*¹⁰. Free energy estimates were made using the method of Tinoco *et al.*¹⁸.



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Occurrence of *trans*-ribosylzeatin in *Agrobacterium tumefaciens* tRNA

THE cytokinin-active N-6-isoprenoid-substituted purine nucleosides have wide distribution as minor components of the tRNA of animals, bacteria and plants. Skoog, Hall and others (refs in ref. 1) have shown that there are three classes of cytokinin nucleosides, and that one member of each predominates in the tRNA of organisms from each kingdom. Thus, animal tRNA contains 6-(3-methylbut-2-enylamino)-9- β -D-ribofuranosyl purine² (iPA); whereas in bacterial tRNA, the purine ring is thio-methylated and the predominant cytokinin-active nucleoside is 6-(3-methylbut-2-enylamino)-2-methylthio-9- β -D-ribofuranosyl purine³ (ms-iPA). In plants, the isoprenoid side chain is hydroxylated and the predominant cytokinin-active nucleoside is 6-(4-hydroxy-3-methylbut-2-enylamino)-9- β -D-ribofuranosyl purine⁴ (zeatin riboside, ZR). Minor amounts of 2-methylthio zeatin riboside (ms-ZR) are also present⁵. But the distribution of these nucleosides may not be quite so clearcut. The plant pathogenic prokaryote *Corynebacterium fascians* has been shown⁶ to release *cis*-zeatin into the culture medium. Other evidence⁷ suggests that *Pseudomonas aeruginosa* tRNA contains ms-ZR. It may be that some prokaryotes have acquired the ability to hydroxylate the isoprenoid side chain and that zeatin and its derivatives are not restricted to plants. We have now examined the cytokinin-active nucleosides from the tRNA of *Agrobacterium tumefaciens*, the causative organism of plant crown gall disease. Besides the expected ms-iPA and small amount of iPA, *trans*-ZR was present in significant amounts.

Four strains of *A. tumefaciens* were examined. Two strains, IIBV7 and C-58, are virulent and have been shown to harbour a large plasmid which is associated in some way with virulence⁸. The other two strains IIBNV6 and NT1, are avirulent and do not contain the plasmid. Strains C-58 and NT1 are very closely related in that NT1 is derived from C-58 by heat treatment which causes elimination of the plasmid with concurrent loss of virulence.

Cells were grown to late log phase on rich medium⁹. The tRNA was isolated by Kirby's procedure, purified by extraction with sodium acetate, and freed from traces of DNA by treatment with *m*-cresol-sodium benzoate¹⁰. Cytokinin-active nucleosides were isolated by alkaline hydrolysis and dephosphorylation with *E. coli* alkaline phosphatase and were extracted into 1-butanol. The butanol-soluble fraction was subjected to high pressure reversed-phase chromatography (HPLC) on octadecyl-substituted silica and cytokinin activity was detected in the column eluate by means of the cucumber cotyledon bioassay of Fletcher and McCullagh¹¹. Figure 1 shows an HPLC profile obtained for IIBNV6 tRNA hydrolysate.

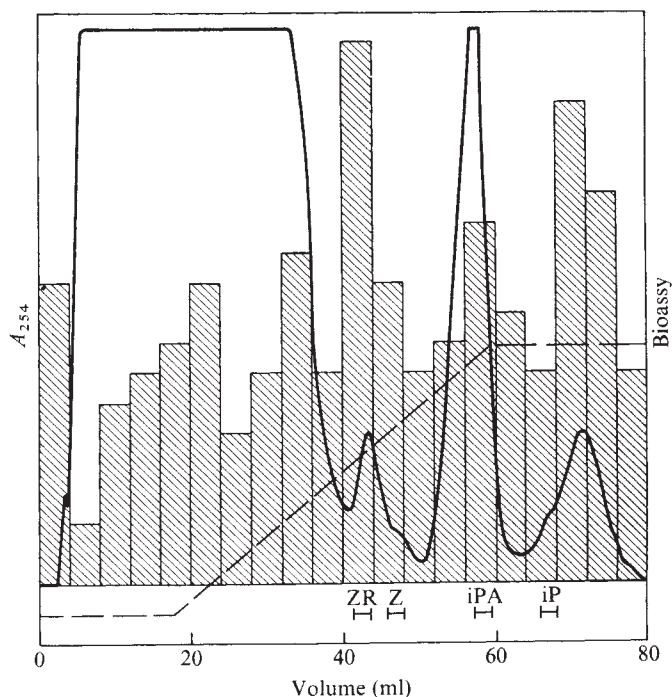
Standard cytokinins eluted from the column in decreasing order of polarity. The most polar, zeatin riboside, eluted before zeatin which in turn eluted before iPA, iP and ms-iPA. The hydrolysate contained five regions where cytokinin activity was significantly above baseline. The first two peaks of activity eluted

at 16–24 ml and 32–36 ml and contained substances more polar than zeatin riboside. They have not been characterised further. The three major peaks (eluting at 40–48 ml, 56–64 ml and 68–76 ml) coeluted with authentic ZR, iPA and ms-iPA. To establish their identity, the appropriate fractions were pooled and the nucleosides were converted into their permethyl¹² or trimethylsilyl (TMS) ethers¹³ which were then examined by gas chromatography-mass spectroscopy (GC-MS).

The fraction with mobility identical to iPA gave, when permethylated, a derivative which exhibited a single peak on gas chromatography identical in retention time to permethylated iPA. Examination of the mass spectrum of this peak showed it to possess a molecular ion $M^+ = 391$ and major fragment ions at 376 (loss of CH_3^+), 348 (loss of $C_3H_7^+$ with ring closure of the isoprenoid side chain onto the purine N-1 position), 217 (fission of the base and ribose with transfer of a hydrogen atom to give BH^+), 202 (loss of CH_3^+ from BH^+), and 174 (loss of $C_3H_7^+$ from BH^+). The ion at $m/e = 45$ was characteristic of the fragment ion $CH_3OCH_2^+$ arising from the 6 position of ribose¹². Studies with deuterated methyl iodide confirmed the fragmentation assignments and the presence of four methyl groups within the molecule. Its spectrum was identical with an authentic sample of tetramethyl iPA. Von Minden and McCloskey¹⁵ have reported an identical spectrum and peak assignments for tetramethyl iPA.

Similarly, the HPLC fraction corresponding in mobility to ms-iPA gave on permethylation a product whose GC retention

Fig. 1 High pressure liquid chromatography of *A. tumefaciens* (strain IIBNV6) tRNA hydrolysate. Purified tRNA ($\sim 2,000 A_{260}$ U from organisms grown in rich medium to late log phase was hydrolysed with 0.3 M KOH under nitrogen at 37 °C for 24 h, treated with bacterial alkaline phosphatase (Sigma, 150 U ml⁻¹) at pH 8.8 in the presence of 10 mM MgCl₂ at 37 °C for 16 h and extracted with 1-butanol. The extract was evaporated, taken up in a minimum volume of 5% ethanol, pH 3.5, and applied to a 0.3 $\times$ 60-cm column of octadecyl-substituted silica (C₁₈/Porasil B, 37–75 μ m, Waters Associates) previously equilibrated with 0.02 M ammonium acetate buffer, pH 3.5, containing 5% ethanol. The column was eluted with a linearly increasing gradient of ethanol (5–50%) over 40 ml at a flow rate of 4 ml min⁻¹. Fractions (4 ml) were collected, lyophilised and assayed for the presence of cytokinin-active nucleosides by the procedure of Fletcher and McCullagh¹¹. ---, Ethanol gradient; —, A_{254} . Hatching shows cytokinin activity (arbitrary units). Cytokinin standards eluted at the positions indicated.



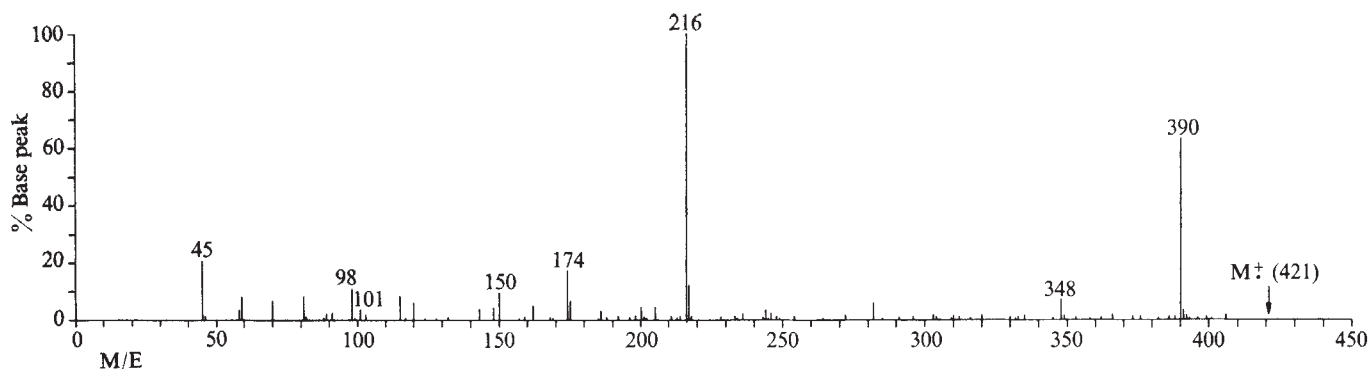


Fig. 2 Mass spectrum of permethylated nucleoside from *A. tumefaciens* tRNA. HPLC fractions containing cytokinin-active material with the same mobility as ZR (Fig. 1 fractions 40–48 ml) were evaporated and the residue was permethylated by reaction with methyl iodide in the presence of a fivefold excess of dimethyl-sulphinyl anion in dimethylsulphoxide¹². The reaction mixture was diluted with water, the permethyl ethers were extracted into chloroform and analysed by GC-MS. GC conditions were: glass column; 3% Dexsil on Anachrom A, 28 cm; He carrier, flow 15 ml min⁻¹; 150 °C for 3 min, then 8 °C min⁻¹ to 350 °C. The column effluent was admitted to the source of a Varian CH7 magnetic sector spectrometer through a single stage glass jet separator at 240 °C. Spectra were recorded continuously (at 70 eV) during the chromatographic run and processed, stored, and analysed on an accompanying data system (System Industries; System 150).

time and mass spectrum were identical with those of authentic tetramethyl ms-IPA. $M^+ = 437$, fragment ions at m/e 423 ($-\text{CH}_3^+$), 394 ($-\text{C}_3\text{H}_7^+$ and side chain cyclisation), 263 (BH^+), 248 ($\text{BH}^+ - \text{CH}^+$), and 220 ($-\text{C}_3\text{H}_7^+$ from BH^+). As expected, all major fragments derived from the adenine nucleus were shifted +46 mass units from the corresponding iPA fragments due to the presence of the thiomethyl group at the 2 position of the purine ring.

The HPLC fraction with mobility equal to that of ZR gave on permethylation a product which GC revealed to have two major components. The first gave a mass spectrum identical to authentic permethylated dimethyl adenosine and was not examined further. The second, whose spectrum is shown in Fig. 2 had major fragment ions present at 390 and 216, and a small molecular ion at 421. Its spectrum was identical to that of authentic pentamethyl ZR. Probable peak assignments (confirmed by deuteromethylation) are $M^+ = 421$; 390, loss of OCH_3 ; 216, loss of OCH_3 from BH^+ ; 174, loss of C_3H_7 from BH^+ (cyclisation). Loss of the allylic methyl ether fragment (OCH_3) was favoured over the loss of C_3H_7 and subsequent ring closure found in iPA and ms-IPA. Details of these spectra will be published elsewhere.

Examination of the trimethylsilyl ethers derived from this HPLC fraction confirmed the presence of ZR. Gas liquid chromatography on Dexsil 300 can resolve *cis*-(TMS)₄ZR from *trans*-(TMS)₄ZR (ref. 13). The sample of (TMS)₄ZR from IIBNV6 hydrolysate had a retention time of 18.2 min, identical to that of authentic *trans*-(TMS)₄ZR. The *cis*-isomer in identical conditions had $R_t = 17.8$ min.

We conclude, therefore, that *A. tumefaciens* strain IIBNV6 tRNA contains, besides the iPA and ms-IPA expected for prokaryotes, the hydroxylated cytokinin nucleoside *trans*-ZR, the *cis*-isomer of which is normally found in plant tRNA. Other strains of *A. tumefaciens* also contained *trans*-ZR although at lower levels than IIBNV6. Visual estimates (from HPLC and GC traces) suggested that *trans*-ZR made up about 30% of the total cytokinin-active nucleosides in IIBNV6 tRNA. In the related virulent strain IIBV7 the level was comparable. In C-58 and NT1, however, the level was much lower (5–10% of the total) and a fourth active component was present. It had a mobility on HPLC intermediate between ZR and iPA, and has yet to be identified. Differences in cytokinin content between strains did not appear to correlate with virulence and may reflect the nutritional and growth status of the culture, as is the case for the modified nucleoside "Q" in BALB/3T3 tRNA (ref. 16).

The unequivocal demonstration of zeatin riboside in the tRNA of *A. tumefaciens* confirms evidence developed by others that some prokaryotes may be able to hydroxylate the isoprenoid sidechain of cytokinins. Cheryail⁷ used ³⁵S to demonstrate the

probable presence of methylthiozeatinriboside in *Pseudomonas aeruginosa* tRNA while Torrey¹⁷ has isolated a cytokinin from *Rhizobium* tRNA which, although not identified conclusively, has the chromatographic properties expected for zeatin riboside. In contrast, no evidence has been published for the presence of zeatin riboside in *E. coli*³ or *Staphylococcus epidermidis*¹⁸ tRNA. The common factor which unites those prokaryotes which can hydroxylate the isoprenoid side chain is their association with plants, either as pathogens or symbionts. It remains to be seen whether this factor has a necessary role in their association with the host plant or if it is merely fortuitous.

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matters arising

Scrotal asymmetry: an appendix

TESTICULAR asymmetry, as McManus has shown¹, was a not uncommon feature of Greek and Roman sculpture; the ancient artist, always attentive to nature, seems to have noticed that the right-hand testis is usually the higher, and inferred (apparently incorrectly) that the other ought therefore to be the larger.

For the historian, however, two questions still remain to be answered: when did the Greeks first discover this peculiarity, and how consistently did they observe it in the early stages of their art? McManus's data, coming as it does from museums in Italy, must of necessity be confined almost entirely to sculpture of the classical period onward (that is, from the fifth century BC to Roman times). Since the preceding century, from the birth of monumental sculpture in Greece around 600 BC, was an era of rapid development in both style and understanding of the anatomy of the human body, it may be worthwhile to supplement his

survey with a look at the 80 kouroi, or standing archaic youths, where this feature is preserved intact. I use Richter's typology², based on minute anatomical analyses and generally accepted today, and Harrison's revised chronology³, which puts the beginning of the series at around 600 BC and its end just before 480 BC, the data of the Persian invasion and sack of Athens. The results (Tables 1–3) can be summarised as follows.

As might be expected, the early groups (Sounion and Orchomenos-Thera: Table 1) show a propensity to equalise the testes in both height and size (or, occasionally, to model them as one; this is particularly the case with statuettes, and continues, though to a diminishing extent, down to the end of the Archaic period: see ref. 2, nos. 13, 22, 26, and so on: in such cases I record the two as "equal") though even at this early date the right is already higher than the left in about two-fifths of the statues sampled. In these and the six statues in which the opposite case obtains, the distribution of size seems to be more-or-less random.

By the middle of the century (Tenea-Volomandra and Melos groups: Table 2), the right testis is the higher in a majority of cases, though in only half of these is the other the larger: the tendency to equalise the testes in size is still dominant.

In the final period (Anavyssos-Ptoon 12 and Ptoon 20 groups: Table 3) the classical scheme is already established. Again, the right testis is usually the higher (though about a quarter of all sculptors, intent on symmetry, still persist in making the two equal in height and size—a practice that is, apparently, to continue), (see ref. 1, Table 1), and the left is now regularly the larger. Significantly, in no case now is the higher testis the larger: Greek sculptors seem to have bowed to the dictates of common sense, as opposed to science, even in the adolescence of their art.

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Table 1 Analysis of the scrotal asymmetry of 26 statues from Richter's Sounion and Orchomenos-Thera groups (c.600–c.570 BC)

		Side of higher testicle			Total
		Left	Equal	Right	
Side of larger testicle	Left	3		6	9
	Equal		10	1	11
	Right	2		4	6
	Total	5	10	11	26

Table 2 Analysis of the scrotal asymmetry of 29 statues from Richter's Tenea-Volomandra and Melos groups (c.570–c.540 BC)

		Side of higher testicle			Total
		Left	Equal	Right	
Side of larger testicle	Left			9	9
	Equal	1	8	7	16
	Right	2		2	4
	Total	3	8	18	29

Table 3 Analysis of the scrotal asymmetry of 25 statues from Richter's Anavyssos-Ptoon 12 and Ptoon 20 groups (c.540–c.480 BC)

		Side of higher testicle			Total
		Left	Equal	Right	
Side of larger testicle	Left			13	13
	Equal	1	6		7
	Right	5			5
	Total	6	6	13	25

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Specificity of transfer factor

THE letter by Salaman and Valdimarsson¹ is an important step forward in the understanding of the problem of whether the activities of dialysable transfer factor (TFd) are specific or not. They admit that two substances may be involved in the effects of dialysable leukocyte extracts (DLE)—one adjuvant-like material and another specific substance (that is, TFd). In fact, until recently, reports on the effects of TFd dealt with the effects of crude preparations of DLE or roughly purified fractions thereof.

There remains, however, one important point which, although sug-

gested by Salaman and Valdimarsson when they reported that nonspecific effects are more evident in tuberculin-positive than tuberculin-negative individuals, is generally overlooked and which contributes to the confusion in this area of research. It was formerly claimed by medical practitioners that there were no "diseases", only "patients". In other words, a given disease may have a different clinical course, before and/or during treatment, in different patients. This raises the question of recipient peculiarity or, in the case of DLE, "recipient specificity". The effects of DLE are also surely dependent on the specific characteristics of the recipient; for example, it can often be observed that, no matter how strongly positive a leukocyte donor is to a given set of antigens by skin testing, the DLE preparation obtained induces positive skin-test reactivity only to one of the antigens in some recipients and only to another in other recipients, and some show increased lymphocyte DNA synthesis in the presence of antigens *in vitro* whereas others do not²⁻⁴. Therefore, investigators who intend to study the effects of DLE should consider (at least until it is clearly demonstrated to the contrary) that they are probably dealing not only with an adjuvant-like material and a donor-specific substance (TFd) but also, in both cases, with recipient specificity, which can mask the effects of these substances—if indeed they exist.

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CSF^{3,4}. Part of the high concentration of protein, however, may have been due to hypercapnia, since foetuses obtained from abortions are likely to be asphyxiated by the time CSF samples are taken. Hypercapnia increases the penetration of albumin⁵ and other lipid insoluble molecules⁶ from blood into CSF. This is suggested by the very high concentration of CSF protein in the 40-week foetus, compared with normal term newborn infant^{7,8}. Adinolfi *et al.* suggest that their findings confirm the proposition suggested previously by several authors⁹ that the blood-brain and blood-CSF barriers to protein are poorly developed in foetal and perinatal life. An alternative explanation for the high level of protein in foetal CSF, however, is that certain plasma proteins may be transported into foetal CSF at an early stage in brain development^{4,10}. This was based on electron microscopical evidence that the tight junctions (which form the barrier to protein in adult brain¹¹) of cerebral endothelial and choroid plexus epithelial cells are already well developed at a stage when the CSF protein level is very high. There is also evidence⁴ from studies of isotopically-labelled protein that certain plasma proteins (for example, transferrin) may be transported into the CSF of early foetal brain, but not later in development. This finding has recently been supported by studies using human serum proteins detected by immunoassay¹⁰ which showed that both transferrin (molecular weight 74,000) and α foetoprotein (molecular weight 64,000) penetrated from blood into CSF to a much greater extent than albumin (molecular weight 69,000) in 60-d-old foetal sheep—a stage when the CSF contained about 350 mg per 100 ml total protein (term is 147 d).

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Permeability of blood-cerebrospinal fluid barrier during foetal and perinatal life

THE HIGH level of protein in early foetal cerebrospinal fluid (CSF) from human abortion material reported by Adinolfi *et al.*¹ is important confirmation of results obtained from earlier work on human² and animal foetal

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ADINOLFI *ET AL.* REPLY—We thank Saunders *et al.*¹ for calling our attention to their work², and as yet unpublished results³. Regarding the effect of hypercapnia, we do not discount it as a possible factor influencing the levels of plasma proteins in CSF. Saunders *et al.* must be aware of the difficulties of working with human foetal material; it is after all post-mortem material. We stated in our paper⁴ that the results were based on a very selected group of foetuses. Many samples of foetal CSF were discarded because of contamination with blood and almost all foetuses obtained by termination with prostaglandin could not be used.

We acknowledge that the levels of some proteins were higher than expected in the 40-week-old foetus; whether this was due to hypercapnia or other factors we are unable to say. This does not, however, alter our conclusion that the permeability of the blood-CSF barrier is incomplete during foetal life.

Recent evidence, based on the estimations of the levels of specific plasma proteins such as albumin, α -foetoprotein, IgG and prealbumin in serum and CSF from the same foetus, suggests that the mechanisms of transfer vary among different proteins. Thus, for example, whereas the percentage of prealbumin in CSF, as compared with blood, varied between 40 and 70, the levels of other proteins varied from ~ 5 to 20%. These variations were found to be related to the ages of the foetuses.

In our paper⁴ we merely presented our observations; at this stage of our work it seemed to be premature to advance any hypothesis on the mechanisms of transfer of plasma proteins from blood into CSF in human foetuses.

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- ² Durbin, G. M., *et al.*, *J. Physiol., Lond.*, **250**, 25–26P (1975).
- ³ Dziegielewska, K. M., *et al.*, *J. Physiol., Lond.* (in the press).
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reviews

Spirit of Nature

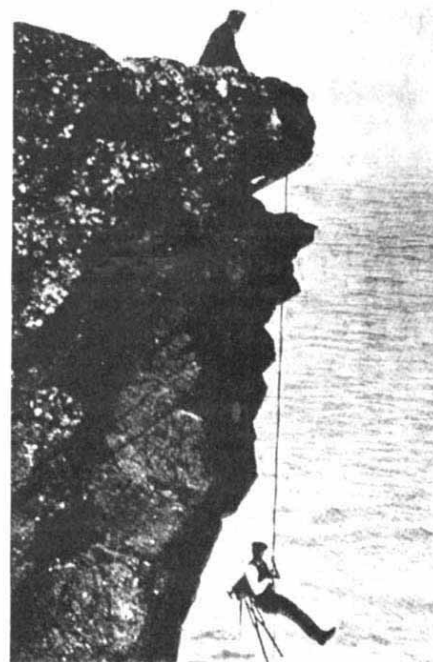
R. Desmond

The Naturalist in Britain: A Social History. By David Elliston Allen. Pp. xii+292. (Allen Lane: London, 1976.) £9.

MR D. ALLEN, taking as his text Emerson's comment that once the facts of natural history are married to human history they become full of life, has attempted the first social account of British natural history. His survey begins with the early seventeenth century when naturalists began exchanging observations and discoveries, occasionally coming together on field excursions such as the 'herborisings' organised by the Society of Apothecaries. Mr Allen has not been able to add much to the little already known about the development of natural history studies in the seventeenth and early eighteenth centuries. By the middle of the eighteenth century the study of natural history was beginning to be accepted as an essential part of a liberal education and this is where Mr Allen's narrative really gets under way. As he points out, natural history attained respectability largely through the researches and teaching of the great Swedish naturalist, Carl Linnaeus. Botany in particular was caught up in the romantic movement which swept Europe. Jean-Jacques Rousseau, a seminal figure in this movement and no mean botanist himself, urged the study of botany as a way of communicating with the essential spirit of Nature.

A great deal of the book is devoted to an examination of the Victorian period when nature study became a popular pastime and was responsible for the many shell pictures and albums of pressed flowers which still survive. It was with evident satisfaction that Charles Kingsley noted that "books of Natural History are finding their way more and more into drawing rooms and school-rooms, and exciting greater thirst for a knowledge which even 20 years ago, was considered superfluous for all but the professional student."

Serious research was fostered by the formation of societies such as the Aurelian Society in 1762, the Linnean Society in 1788, the Horticultural Society of London in 1804 and the



Left, *Photographic gun*, taken from *Le Nature* (1882); right, *bird photography under difficulties*, taken from *Wild Life at Home* by R. Kearton (1899).

Zoological Society of London in 1826. But as Mr Allen convincingly demonstrates, nowhere was enthusiasm and dedication more evident than in the humble gatherings of workingmen naturalists. Such was James Bolton of Halifax who not only taught himself botany but also mastered drawing and engraving to illustrate his books. Stamina and single-mindedness were the sterling qualities so many of these naturalists had in abundance. As a young man William MacGillivray walked 800 miles from Scotland just to see the fine bird collection in the British Museum.

Philip Henry Gosse was so absorbed in his studies that his diary recorded his son's birth almost as a casual afterthought: "Received green swallow from Jamaica. E[liza] delivered of a son." Country parsons stagnating in a cultural desert frequently sought refuge in the world of Nature. One thinks of Gilbert White's meticulous observations at Selborne, M. J. Berkeley's important contributions to mycology, S. R. Hole's delight in roses and the popularisation of natural history in the spate of books by F. O. Morris and J. G. Wood.

Entomology and botany have always been considered an appropriate female occupation, while the collecting of shells was once recommended because they "are so brightly clean, so ornamental to a boudoir." But women's involvement was not always through a gentle pursuit of culture. In the nineteenth century Mrs Gatty and Mrs Griffiths made systematic collections of algae and Mary Anning discovered the first nearly complete skeleton of a *Plesiosaurus* in the Liassic cliffs near Lyme Regis.

Children were introduced to the marvels and mysteries of Nature in books such as E. and S. M. Fitton's *Conversations on Botany* (1817), set out in the familiar form of a catechism between child and parent. Mr Allen only briefly touches on the passion for disseminating knowledge common to all Victorian reformers for whom education was a means to moral improvement. Religious bodies such as the Society for Promoting Christian Knowledge and the Religious Tract Society sponsored a number of primers of botany and zoology. Probably the limitations of space prevented Mr Allen from giving due weight to

the important role of some commercial publishers, notably Richard Groombridge and Lovell Reeve, in promoting the production of inexpensive natural history books.

With the advancement of serious research in the universities and the emergence of new disciplines such as ecology, the gap between the professional and the amateur has inevitably widened. Nevertheless, the appeal of natural history has always been the scope it affords the amateur. Mr Allen is particularly good in explaining its increasing popularity during the present century, due in part, he maintains, to the rediscovery of the countryside by bicycle and motor-car. It is now no

longer a matter for surprise when books like the Rev. W. Keble Martin's *Concise British Flora in Colour* (1965) become instant bestsellers.

Mr Allen is a keen field botanist and it is therefore understandable that he gives a fuller treatment to botany than to zoology. Nonetheless he has unearthed much new or forgotten material from a vast assortment of printed and manuscript sources to compile this fascinating story of the discovery of Nature, and of the men and women who devoted their lives to its study. □

Mr Desmond is President of the UK Society for the Bibliography of Natural History.

Changing attitudes to wildlife

Nature in Trust: The History of Nature Conservation in Britain. By John Sheail. Pp. xiv+270. (Blackie: Glasgow and London, May 1976.) £5.95.

BEGINNING with a short survey of man's attitudes to nature preservation in the sixteenth century, Dr Sheail has traced, during the years for which he has the most complete documentation, the changing attitudes to the protection of wildlife. When naturalists first thought about protection they concentrated on preserving rare and distinctive plants and animals by means of legislation. Later, as the science of ecology developed, the concept of habitat protection prevailed. But having come to this conclusion land purchase was still not straightforward. The Society for the Promotion of Nature Reserves (SPNR) came into existence to promote the purchase of land by others, particularly the National Trust, and did not in those early days have money itself.

This book is a recital of facts gathered from the archives and files of voluntary bodies, particularly the SPNR and the Royal Society for the Protection of Birds (RSPB), and from governmental organisations. The bulk of the material relates to the first half of this century and chiefly between 1920 and 1950. It records the activities of the various committees which were set up, or which set themselves up, to examine the types of land which required protection, the different ways of acquiring it, the setting up of scientific authorities to manage the reserves, to undertake research, to advise governments and others on scientific problems.

At one point in the 1930s no one had clear ideas of what they wanted—what, for instance, the relationship should be between national parks and nature reserves, and the Government gave up trying to solve the problem. Although

thought was given to environmental conservation in post-World War I reconstruction planning it fell to the voluntary bodies once again to persuade the Government to take action. Ultimately it was the committee headed by Sir Julian Huxley and the Nature Reserves Investigation Committee which began to produce the kind of recommendation that brought nature conservation out of the national park sphere of influence and that led to the establishment of the Nature Conservancy with its original triple function of reserve acquisition, research and scientific advice. Dr Sheail finally records briefly what has been happening since the 1950s.

I was enormously impressed by the facts that Dr Sheail has collected together and incorporated in his book. I wondered whether it would not have been possible to have digested them further or discussed their implication. He writes in an easy manner but at times I found it difficult to follow all the threads. The other point which rather worried me was that the book is described as *The History of Nature Conservation in Britain*. It is, however, more of a history of the development of the organisation side of nature conservation. There is no real discussion on the effect of writers, broadcasters and others who influenced the public to take a deeper interest in nature conservation, and various other activities.

I feel that the full history of nature conservation has still to be written. In saying this I am not denigrating what has been done in this volume. It is an enormous work of scholarship on one major aspect of the growth of conservation. It is readable and will be of great value and interest. As a history it has lessons for the present.

Peter Conder

Peter Conder has recently retired as Director of the UK Royal Society for the Protection of Birds.

Resistance in *Pseudomonas*

Resistance of Pseudomonas aeruginosa. Edited by M. R. W. Brown. Pp. ix+335. (Wiley-Interscience: London and New York, September 1975.) £14.

THIS book is intended to define the problems associated with drug-resistant *Pseudomonas aeruginosa* from a number of different angles, in chapters written by different authors. It suffers from the inevitable unevenness and repetition that result from diverse authorship in discussing a narrow field. The repetition is tiresome and it is clear that the editor did not exercise sufficient control over his team. For example, chapters 4 and 5 present four electron micrographs in common, one of which is inverted in one chapter in relation to the other. The action of EDTA on *Pseudomonas* is mentioned too frequently. The chapter on antibiotic inactivation and its genetic basis carries some curious statements. For example, there is confusion between the spread of resistance plasmids and that of pathogenic bacterial clones carrying them. Intestinal bacteria other than *Pseudomonas* are very loosely referred to as "enterics" in this chapter. It also uses the term "evolution" imprecisely, and presents the puzzling statement that "there is good evidence that two clones of Gram-negative bacteria specifying resistance to gentamicin, including Pseudomonads, have become widespread in France". If there is more than one Pseudomonad there is no room in two clones for other genera. And if there are one or more genera of bacteria other than Pseudomonads there is no room for a plurality of Pseudomonads.

The chapter on epidemiological typing presents details of a phage-typing scheme for *Pseudomonas* in such a way that no-one would realise that the principles and techniques it enunciates owe their origin and even terminology to the phage-typing schemes for *Salmonella typhi* and other salmonellae, none of which is mentioned. It seems rather late in the day to publish a photograph of a surface spot titration of phage (Fig 1, p196); and Fig 2 on the same page is a poor illustration of a phage-typing result.

Although valuable data are presented, there are faults, overstatements and omissions in all eight chapters of this book. All things considered it is of doubtful value at a price of £14.

E. S. Anderson

Red colobus

The Red Colobus Monkey. (Wildlife Behavior and Ecology.) By Thomas T. Struhsaker. Pp. xiv+311+37 plates. (University of Chicago: Chicago and London, 1975.) £15.

ADEQUATE descriptions of the behaviour and ecology of rain forest mammals are rare, largely because difficult observations conditions impose severe restrictions on the scope and accuracy of the data which can be collected. Struhsaker's study of the red colobus is one of the few cases in which an observer has succeeded in producing a comprehensive survey both of social behaviour and feeding ecology, and represents a major achievement in this field.

The four main chapters cover population dispersion, social behaviour (agonistic behaviour, grooming, sexual behaviour, mother-infant relationships and intragroup dispersion) and feeding ecology (food selection, ranging behaviour and interspecific rela-

tions). On each topic the author has been at pains to include both detailed qualitative descriptions of the behaviour patterns and, where relevant, quantitative material concerning their frequency.

The book is of particular interest because of the comparisons which it draws between the red colobus and the partially sympatric black-and-white colobus. The two species differ markedly in grouping patterns, red colobus living in large, multi-male troops which occupy extensive home-ranges—black-and-white in smaller and more cohesive single-male troops in defended territories. The study shows that these differences are associated with variation in dietetic diversity and in the proportion of mature leaves in the diet at particular times of year.

Unlike its three precursors in the same series, this monograph is clearly aimed at specialists and is not easy to read, partly because the text relies heavily on 76 pages of tables published at the end of the volume.

T. H. Clutton-Brock

Chromosome configurations at meiosis

Meiotic Configurations: A Source of Information for Estimating Genetic Parameters. (Monographs on Theoretical and Applied Genetics, Vol. 1.) By J. Sybenga. Pp. x+251. (Springer: Berlin and New York, 1975.) DM68; \$27.90.

DR SYBENGA gives an account of the different kinds of chromosome configurations encountered at meiosis; how they vary in response to change in genotype and environment, and to change in chromosome structure and number. He presents a critical survey of statistical methods by which the frequencies and distributions of the various kinds of configurations within and among individuals may be efficiently described and analysed. Many of the methods concern variation in configurations dependent on chiasma frequencies and distribution. Dr Sybenga is evidently sceptical of the ability of cytologists to score chiasmata with accuracy even in organisms, such as rye, with large chromosomes. The scepticism, or modesty, embraces Dr Sybenga himself who, by way of caution, prefers to score the number of chromosome arms bound per bivalent rather than the number of chiasmata per arm. I think Dr Sybenga is over modest. I know many who claim to score chiasmata

with a very high degree of accuracy and thereby acquire more detailed information about the consequences of variation in chiasma formation!

To date, the emphasis in this field of investigation is of course on the consequences of variation in chiasma formation. We are still a long way from understanding the *mechanics* of chiasma formation or of other events at meiosis such as chromosome pairing. Many of the facts presented in this book provide clues as to why experiments seeking to elucidate the mechanisms are, to an unusual degree, difficult and inconclusive. Consider, for example, the question of establishing to what extent a structural change within a chromosome affects its ability to pair and form chiasmata with its homologous partner. We may introduce an alien chromosome segment, by transposition, and observe its effects. The trouble arises in interpreting these effects because the structural change is inevitably accompanied by a change in the chromosome genotype—that is in the content and arrangement of genes whose expression in itself affects pairing and chiasma formation independently of structural homology. The effects of genotype and of structure are here, typically, confounded. Were we able to distinguish between them we would make some progress.

Dr Sybenga, who has specialised in the study of meiosis, has produced a useful book, bursting with facts and figures which are presented with clarity and authority.

H. Rees

Life and death of microbes

The Survival of Vegetative Microbes. (Twenty-sixth Symposium of the Society for General Microbiology, April 1976.) Edited by T. R. G. Gray and J. R. Postgate. Pp. x+432. (Cambridge University: Cambridge and London, April 1976.) £12.

THIS year's Society of General Microbiology Symposium dealt with the subject of Survival of Vegetative Microbes and this book arises from it.

Death of microbes is a subject which impinges on many areas of microbiology but relatively little interest has been shown in such a 'vital' theme. This book brings together a number of chapters on this topic in an attempt to stimulate interest in this important area.

Professor Postgate introduces the subject in an admirably well written first chapter. Several of his comments on the problems of working in this field should be taken to heart by microbiologists, not least by some of the later contributors to the book. The next chapter by Professor Dawes is a classic review on the role of endogenous metabolism on survival of microbes.

The effect of other physiological stresses on survival are dealt with in a series of chapters on cold-shock and freezing, drying and aerosol stress, osmotic stress, ultraviolet and ionising radiation, light and heat. The next group of chapters deal with the survival of microbes in natural environments. Professor Morita's chapter deals with survival (in the sea, particularly) in conditions of extreme pressure. There then follows chapters on survival *in vivo* and in the soil. The last two contributions deal with the specific role of thymidine starvation, and the effect of chemicals on survival.

Any book which is a collection of papers from different authors would be expected to vary in standard. This is indeed the case but the best are of a very high standard indeed. The two editors are to be congratulated in producing such a readable and informative book, and it is to be hoped that it will stimulate interest in this neglected area.

I strongly recommend that this book be general reading for all microbiologists so that they may appreciate the role that death as well as life may play in their areas of interest.

D. C. Ellwood

An Introduction to Electromagnetic Fields. By R. L. Ferrari. Pp. xi+202. (Van Nostrand Reinhold: New York and London, September 1975.) Cloth £7; Paperback £3.95.

THIS is a slim volume which forms a welcome addition to the stock of available texts on electromagnetic fields. Intended for an undergraduate audience, it uses extensively familiar examples of electric and magnetic phenomena to arrive at a clear physical understanding of field theory. Comprehension is also considerably assisted by the liberal use of line drawings and illustrations. Although the mathematical level of this book is comparable to others intended for the same audience, Ferrari's distinguishes itself by not forcing the mathematical pace, but rather by making full use of mathematics as a manipulative tool to assist in physical reasoning. It is well written and eminently readable. As a basis for a course of lectures, it would be desirable to supplement it by a well-chosen reading list of other books and articles, enabling the student to delve more deeply into areas of interest to him, and to pursue in some depths individual topics. If its brevity can persuade a growing circle of engineering students to reach beyond a single volume to study a subject, rather than merely a book, it will have rendered great service.

P. Silvester

Methods of Optimisation. By G. R. Walsh. Pp. x+200. (Wiley-Interscience: London, September 1975.) Cloth £7.50; Paper £3.75.

THIS text essentially reviews a number of optimisation techniques, more from the algorithmic point of view than that of attempting a unified theoretical picture, but with enough theory given that the reader can establish a certain perspective. In this spirit it runs through Lagrangian methods, non-linear programming (Kuhn-Tucker conditions, quadratic programming), search and gradient methods of optimisation (these sections quite thorough), some special techniques of constrained numerical optimisation, and a study of some special problems in dynamic programming. In treating Lagrangian, Kuhn-Tucker and duality theory, the author regrettably never gets away from local versions of the results, and also misses their geometrical significance, which is so simplifying and illuminating. As a medium-level no-nonsense introduction, however, the book is quite attractive.

P. Whittle

Animal Migration and Navigation. By Philip Street. Pp. 144. (David and Charles: Newton Abbot, London and Vancouver, March 1976.) £4.95.

A POPULAR book such as this should ideally present a reasonably accurate account of the current state of knowledge in a readable and interesting style. Philip Street's book is certainly readable, but it is sadly inadequate as an account of modern work on migration and navigation. The author often digresses from the advertised subject matter—for example, to discuss infrared eyes in snakes, echolocation of prey in bats and communication in honeybees. Much of the rest of the book consists of a catalogue

of examples of long distance migrations in fish, marine mammals, amphibians, insects and birds. Although in his introduction, the author asserts that long distance navigation is one of the most fascinating aspects of animal behaviour, the book says very little about navigation (the term itself is never explained) and most of the work described is now out of date. For example, the chapter on bird migration and navigation discusses pigeon homing without mentioning any work more recent than about 1955, and the account of stellar orientation refers only to the early work of Sauer. One does not expect a popular book to be right up to date, but any such book should not be as outdated as this one.

John Krebs

Introduction to Liquid Crystals. Edited by E. B. Priestley, Peter J. Wojtowicz and Ping Sheng. Pp. xi+356. (Plenum: New York and London, 1975.) \$27.

THIS book derives from seminars given at RCA Laboratories in Princeton in 1973 and it is strongly polarised, theoretically and practically, towards the technology of using liquid crystals in electro-optical devices. The preface states this, but the book title is potentially misleading to those expecting an introduction to liquid crystals as a whole. The book is also mainly concerned with the physics of the relevant liquid crystals (nematic, cholesteric, smectic A); the chemical input is small, and, disappointingly,

no analysis of the relative chemical merits of available liquid crystal materials emerges. The book is, however, a valuable and timely text on the physics of liquid crystals in relation to their use in displays and one which will benefit all concerned with this technology.

Being multi-authored, the 18 chapters vary in quality; those on nematic order, continuum theory, electrohydrodynamic instabilities, Landau-de Gennes theory of phase transitions, and the packaging and addressing techniques for liquid crystal displays are very good. The end chapter on lyotropic liquid crystal systems and membranes points to a growing awareness of the possible technological scope for these types of system.

References to the 1974 literature are quite numerous. The presentation of the text, diagrams and equations is clear, and the index provided is adequate.

G. W. Gray

The Seismics of Heterogeneous and Turbid Media. By A. N. Nikolaev. Translated from Russian. Pp. iv+140. (Israel Program for Scientific Translations: Jerusalem; Wiley: Chichester, February 1976.) £8.25; \$16.50.

THIS is a study of the theory and observations of small-scale variations in the elastic parameters (the turbidity) of the Earth. Fundamental questions arising from the definition of "small-scale", and from the treatment of such variations as random, are unfortunately left largely unresolved; the underlying theory is based on the scalar wave equation and the reader is merely referred to the literature on the question of validity. Ultimately the author defines a "turbidity factor" which is to be integrated along a seismic ray to get the mean square variation in log amplitude—a formula quaintly described as "usually invalid" but "nevertheless . . . useful". Regrettably it is noted later on that its validity is not yet verified by experiment. Some model experiments, however, are described and the design of field experiments and analysis of certain observations are given in some detail. The net results are contained in plots of turbidity against depth for sections of one continental and three oceanic crusts, leaving the reader somewhat in the dark as to the related physical properties of the medium. The book is very hard to read as the result of a rather ham-fisted translation.

J. A. Hudson

Books brief